ESSENTIALS OF HUMAN REPRODUCTION

Clinical Aspects Normal and Abnormal

CONTRIBUTORS

ROBERT W BROWN M D
HARVARD MEDICAL SCHOOL *and* BOSTON UNIVERSITY SCHOOL OF MEDICINE

BARBARA J CALL M D
PETER BENT BRIGHAM HOSPITAL

JEANNE A EPSTEIN M D
NEW YORK UNIVERSITY SCHOOL OF MEDICINE

ROBERT H FENNELL JR M D
UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE

CELSO RAMON GARCIA M D
HARVARD MEDICAL SCHOOL *and* THE ROCK REPRODUCTIVE STUDY CENTER
FREE HOSPITAL FOR WOMEN

ROBERT W KISTNER M D
HARVARD MEDICAL SCHOOL *and* THE FREE HOSPITAL FOR WOMEN

KERMIT E KRANTZ M D
UNIVERSITY OF ARKANSAS SCHOOL OF MEDICINE

HERBERT S KUPPERMAN M D PH D
NEW YORK UNIVERSITY SCHOOL OF MEDICINE

LUIGI MASTROIANNI JR M D
YALE UNIVERSITY SCHOOL OF MEDICINE

WILLIAM B OBER M D
COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS
and ALBERT EINSTEIN COLLEGE OF MEDICINE

DORIS PHELPS PH D
VANDERBILT UNIVERSITY SCHOOL OF MEDICINE

JOHN ROCK M D
HARVARD MEDICAL SCHOOL *and* THE ROCK REPRODUCTIVE STUDY CENTER
FREE HOSPITAL FOR WOMEN

JOSEPH THOMAS VELARDO PH D
YALE UNIVERSITY SCHOOL OF MEDICINE

ROBERT C WABNITZ MED ILLUS
ST CLARE'S HOSPITAL OF NEW YORK

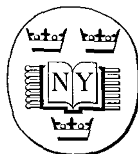
ESSENTIALS OF HUMAN REPRODUCTION

Clinical Aspects, Normal and Abnormal

EDITED BY

JOSEPH THOMAS VELARDO

YALE UNIVERSITY SCHOOL OF MEDICINE



NEW YORK

OXFORD UNIVERSITY PRESS

1958

Library of Congress Catalogue Card Number 58-9177

PRINTED IN THE UNITED STATES OF AMERICA

v

DEDICATED TO OUR TEACHERS

George W Corner

Roy O Greep

Carl G Hartman

THEIR SCHOLARLY ACHIEVEMENTS PRESENT
AN EXACTING FRAMEWORK FOR THE CLINICAL
INVESTIGATION OF HUMAN REPRODUCTION

PREFACE

Medical research concerned with all phases of human reproduction is making great progress It has been almost twenty years since a definitive account of the essentials of human reproduction has been related in textbook form Therefore a textbook dealing with the several aspects of human reproduction is quite timely The world while growing in population is becoming smaller in concept especially since it is now possible to travel around the world in less than one full day It seems that we have learned a great deal about so very much outside of the biological universe that it now appears more than worthwhile to take account of ourselves an account of our biologic and clinical manifestations Perhaps there is no better place from which to start than the endocrine mechanisms of human reproduction

The primary purpose of this book is to relate the several aspects of human reproduction In this aim it is most appropriate to begin with (1) an appraisal of the status of our knowledge (2) the general principles of reproductive endocrinology and (3) the more clinical aspects of endocrine mechanisms associated with reproduction The ten chapters comprising this book unfold with a thoroughly documented discussion of the source of new potential life ovulation Following this there are at least two pathways pregnancy or menstruation In addition to a description of the normal aspects of human reproductive mechanisms the abnormal conditions from infertility pregnancy complications and frank pathology of the male and female reproductive systems are described In this approach it becomes possible to describe the whole biologic career of the reproductive system as well as its variants Although brief in nature the present work is intentionally innocent of sweeping generalizations undue oversimplifications and tacit assumptions The text is further characterized by the inclusion of numerous pertinent illustrations and an evaluation of the most recent clinical literature on reproductive mechanisms

The editor and contributors are indebted to the numerous people who made so many of their clinical cases and lifetime studies available for inclusion in the present book We are particularly grateful to Professors William U Gardner and John F Fulton for their steady and unselfish assistance from the inception of this project Messrs James W Zarbock and Douglas C Ross Jr Misses Leona Capeless and Katharine Parker and the entire medical department of the Oxford University Press have made this a very pleasant experience The editor owes a great deal of thanks and warm affection to all of his contributors who have worked through numerous drafts and revisions of their chapters Special thanks are due Professors Frederick L Hisaw and Charles M Vaughn for their constant stimulation infectious enthusiasm passion for excellence and for their great part in introducing me to this field of study Finally the editor wishes to express his wholehearted appreciation to his very patient wife Forresta Monica and his family Rose Marie V S F and Arthur and Julia Power for their constant encouragement and understanding during the development writing and editing of this book

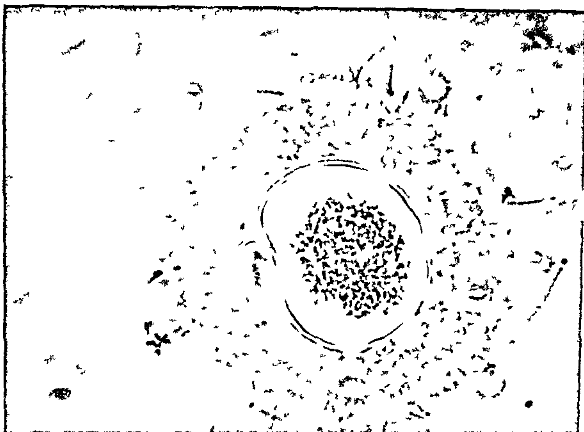
J T V

August 1958
New Haven Connecticut

TABLE OF CONTENTS

ESSENTIALS OF HUMAN REPRODUCTION

Clinical Aspects Normal and Abnormal



LIVING HUMAN EGG AT MOMENT OF FERTILIZATION Numerous spermatozoa in contact with the ovum several penetrating the zona pellucida only one of which will reach the egg nucleus First polar body formed

Courtesy of Dr Landrum B Shettles

I

Endocrines and Reproduction

JOSEPH THOMAS VELARDO PH D

THE PAST twenty years have provided us with many important facts concerning the essentials of human reproduction Previous to this time there was a great deal of speculation Today the advances in mid twentieth century medicine are numerous and we have come to learn a great deal about ourselves and the animal kingdom through comparative studies of reproductive mechanisms [28]

While it is true that basic scientists and clinicians have made great strides in advancing our knowledge of reproductive mechanisms we yet have some serious lamentable lacunae in our present day concepts of human reproduction Like evolution some pieces of information are missing others do not seem to fit yet others may be misleading incorrect or self limiting as a result of the techniques at our disposal But the joining of ranks of the basic scientist and the clinical investigator has resulted in an accumulation of a great deal of new evidence concerning the (1) endocrine physiology of reproduction (2) new and more active synthetic hormones (3) ovulatory process (4) clinical diagnosis and treatment of infertility (5) early detection proper diagnosis and treatment of gynecologic disturbances (6) accurate appraisal of the pathology of the male and female reproductive systems (7) easier childbirth and last but not the least in importance (8) we have uncovered a whole new discipline in steroid biochemistry The present book takes for its central theme an elucidation of the essentials of human reproduction and includes a thorough discussion of the new clinical advances made in the past two decades

What do we really know of the biologic and clinical events of reproduction? Velardo and his associates recently described and evaluated the known biologic data [43] Scientists have made great strides in advancing our knowledge of the biology of reproduction The more clinical aspects of reproductive mechanisms will be explored here It appears very worthwhile to keep in mind while using this book the question What do we really know about ourselves born or unborn?

Mid century medicine has capitalized on the early discoveries and philosophies of Claude Bernard and Walter B Cannon Today we are in a much better position to look and to see more The internal environment and the wisdom of the body have been strong directional pathfinders for all of us in modern medicine We can now appreciate the fact that man is a *whole unit* and any true evaluation must perforce take cognizance of his whole biologic nature This is particularly true of an evaluation of the reproductive process We no longer look to the gonads for *all* of the facts concerned with reproductive failure or success In general it may be said that the gonads the higher biologic centers including the adenohypophysis *and* the hypothalamus the thyroid the adrenal cortex and the biologic and emotional status of the human are all important in an evaluation of reproductive activity

During the period between 1930 and the early 1950s we became fairly convinced that there are pituitary gonadal relationships today it would be more correct to refer to such as hypothalamic adenohypophyseal gonadal inter relationships Recent investigators are pointing most specifically to circumscribed areas of the hypothalamus as being related to gonadotropic thyrotropic and adrenocorticotropic activity [18] Since research in this area is receiving such careful attention by so many competent investigators it seems reasonable to expect that we will witness a clearer concept of these relationships in the foreseeable future for the present however we must be contented with only an indication of the influence of the hypothalamus on reproductive mechanisms

A ENDOCRINE RELATIONSHIPS IN THE FEMALE

The female reproductive system consists of the ovaries the uterine tubes the uterus and the vagina (Fig 1) These are often referred to as the reproductive organs of the internal group and are located within the pelvis The ovaries are commonly called

the paired sex organs of the female whereas the other organs including the external genital structures are grouped together as the secondary (or accessory) sexual organs Comprising the so called external genital organs are the mons pubis the labia majora labia minora the clitoris the bulbus vestibuli and the conspicuous vestibular glands These external structures are located below the urogenital diaphragm and in front of and below the pubic arch While the mammary glands are related to the reproductive system they are not truly to be classified as genital organs The ovaries serve a dual purpose (1) the production of female sex cells ova and (2) the secretion of a complex group of sex hormones the function of which has to do with the growth and development of the associated sexual organs and the mammary glands The secondary sexual structures have three particular functions (1) to provide a receptacle for the male sex cells as well as for their conveyance up to the uterine tubes in the aid of fertilizing the available ovum (2) to provide an environment for the fertilized ovum so that it may successfully develop into an embryo and finally a fetus and (3) to assist in the expulsion of the fetus at the end of gestation

I The Ovarian Hormones

At least four kinds of hormones are associated or implicated with ovarian function estrogens progestational hormones relaxin (plus a substance known as a uterine relaxing factor) and androgens

a Estrogens

Experimental evidence suggests that estradiol 17β is elaborated by the theca interna of the ovarian follicle Estradiol 17β and estrone have been found in the follicular liquor and both of these estrogens can be interconverted one to the other Recently Marrian has shown that estradiol 17β is associated with five metabolites all of the $\Delta^{1,3,5}$ estratriene chemical configuration [27] The suggested metabolic pathway of these estrogens according to Marrian is depicted in Figure 2

Estrogenic action during the normal sequence of events of the human menstrual cycle is associated with the growth phase of the endometrium Furthermore the estrogens are capable of inducing growth of the entire reproductive tract and the mammary glands *The known biologic actions of the estrogens in reproductive mechanisms have been described quite recently by Velardo* [44]

b Progesterone

(Progestational substances progestins)

The corpus luteum is the main endocrine tissue that produces progesterone In addition to progesterone Δ^4 3 ketopregnene 20αol and Δ^4 3 ketopregnene 20βol have been reported to be secreted by the corpus luteum [47] Since it has been most difficult to detect progesterone by known biologic methods much of our information has been based on the

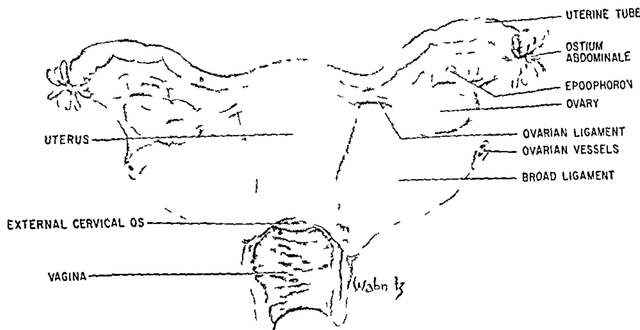


Fig 1 The female reproductive tract (From Velardo J T *The Endocrinology of Reproduction* Oxford University Press New York)

determination of its metabolites pregnanediols or sodium pregnanediol glucuronide (NaPG)

The metabolic career of progesterone is much more complicated than that of estradiol 17β First it should be emphasized that it is very difficult to detect significant amounts of progesterone in the blood stream of the human female As a matter of fact it is practically impossible to detect biologically active progesterone in the blood stream of females within minutes after its exogenous administration While it is impossible to describe the metabolic career of progesterone it may help to examine the C 21 related metabolites of progesterone (Fig 3) [4]

There are many functions ascribed to progesterone but it must be pointed out that in almost every aspect of progestational action the endocrine receptor organ has been influenced by estrogen first The primary functions of progesterone are as follows (a) to induce the typical secretory pattern of the endometrium and therefore cause the uterine glands to produce secretions (b) to prepare the endometrium for nidation of the fertilized ovum and (c) to restrict in part the normal spontaneous contractions of the uterus A detailed account of the biologic actions of progesterone (gestagens) is the subject of a recent review by Velardo [42]

c Relaxin

This non steroidal hormone seems to be a hormone of pregnancy [2] especially since it can be demonstrated only during pregnancy [48] Relaxin has been reported to be effective in the treatment of dysmenorrhea premature labor and threatened abortion Recent clinical experience with a highly purified and potent relaxin preparation revealed that it is quite effective as a pelvic softener softening the pelvic tissues including the cervix vagina and vulva Thus it acts to insure easier childbirth [7]

d Uterine Relaxing Factor

This non steroidal substance although not yet isolated as a separate entity from human source material seems to be chemically and physiologically related to relaxin and is therefore included here for the sake of completeness The basic research on this material indicates that it is a hormone and it has been tested for its clinical efficacy It has been reported to be effective in the treatment of dysmenorrhea premature labor and threatened and habitual abortion [32]

e Androgens

Evidence pointing to the ovaries as a source of androgens is more than circumstantial Several investigators have reported that the ovaries secrete androgens Furthermore it now appears that testosterone may be a precursor of estradiol 17β The Leydig like cells found in the region of the ovarian hila is presumably the source of ovarian androgens [39] Females excrete in their urine approximately 18 to 33 per cent less androgens than do males [15]

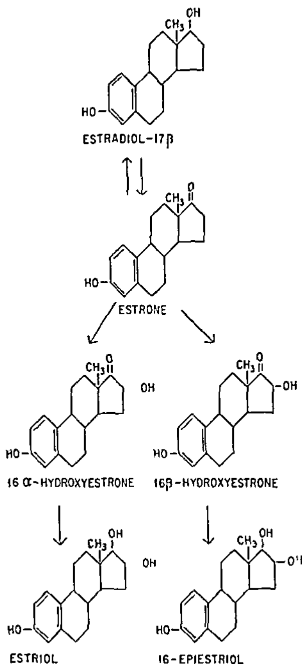


Fig 2 Possible interrelationships of the estrogens determined from human urine (After Marrian G F)

2 The Adenohypophyseal Hormones

As indicated above the adenohypophyseal hormones trigger the ovaries to develop to maturity and se

the paired sex organs of the female whereas the other organs including the external genital structures are grouped together as the secondary (or accessory) sexual organs Comprising the so called external genital organs are the mons pubis the labia majora labia minora the clitoris the bulbis vestibuli and the conspicuous vestibular glands These external structures are located below the urogenital diaphragm and in front of and below the pubic arch While the mammary glands are related to the reproductive system they are not truly to be classified as genital organs The ovaries serve a dual purpose (1) the production of female sex cells ova and (2) the secretion of a complex group of sex hormones the function of which has to do with the growth and development of the associated sexual organs and the mammary glands The secondary sexual structures have three particular functions (1) to provide a receptacle for the male sex cells as well as for their conveyance up to the uterine tubes in the aid of fertilizing the available ovum (2) to provide an environment for the fertilized ovum so that it may successfully develop into an embryo and finally a fetus and (3) to assist in the expulsion of the fetus at the end of gestation

1 The Ovarian Hormones

At least four kinds of hormones are associated *or* implicated with ovarian function estrogens progestational hormones relaxin (plus a substance known as a uterine relaxing factor) and androgens

a Estrogens

Experimental evidence suggests that estradiol 17β is elaborated by the theca interna of the ovarian follicle Estradiol 17β and estrone have been found in the follicular liquor and both of these estrogens can be interconverted one to the other Recently Marrian has shown that estradiol 17β is associated with five metabolites all of the $\Delta^{1,3}$ estratriene chemical configuration [27] The suggested metabolic pathway of these estrogens according to Marrian is depicted in Figure 2

Estrogenic action during the normal sequence of events of the human menstrual cycle is associated with the growth phase of the endometrium Furthermore the estrogens are capable of inducing growth of the entire reproductive tract *and* the mammary glands The known biologic actions of the estrogens in reproductive mechanisms have been described quite recently by Velardo [42]

b Progesterone

(Progestational substances progestins)

The corpus luteum is the main endocrine tissue that produces progesterone In addition to progesterone Δ 3 ketopregnene 20αol and Δ^4 3 ketopregnene 20βol have been reported to be secreted by the corpus luteum [47] Since it has been most difficult to detect progesterone by known biologic methods much of our information has been based on the

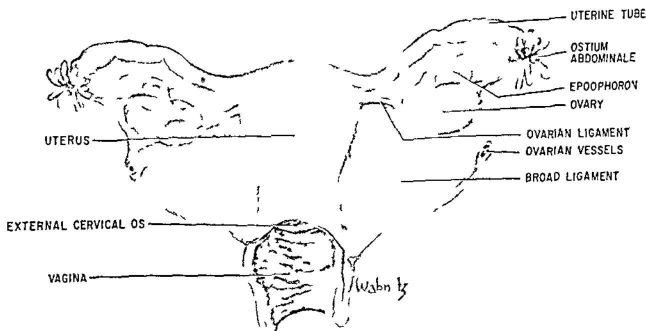


Fig 1 The female reproductive tract (From Velardo J T *The Endocrinology of Reproduction* Oxford University Press New York)

determination of its metabolites pregnanediols or sodium pregnanediol glucuronide (NaPG)

The metabolic career of progesterone is much more complicated than that of estradiol 17β First it should be emphasized that it is very difficult to detect significant amounts of progesterone in the blood stream of the human female As a matter of fact it is practically impossible to detect biologically active progesterone in the blood stream of females within minutes after its exogenous administration While it is impossible to describe the metabolic career of progesterone it may help to examine the C 21 related metabolites of progesterone (Fig 3) [4]

There are many functions ascribed to progesterone but it must be pointed out that in almost every aspect of progestational action the endocrine receptor organ has been influenced by estrogen first The primary functions of progesterone are as follows (a) to induce the typical secretory pattern of the endometrium and therefore cause the uterine glands to produce secretions (b) to prepare the endometrium for nidation of the fertilized ovum and (c) to restrict in part the normal spontaneous contractions of the uterus A detailed account of the biologic actions of progesterone (gestagens) is the subject of a recent review by Velardo [42]

c Relaxin

This non steroidal hormone seems to be a hormone of pregnancy especially since it can be demonstrated only during pregnancy [4] Relaxin has been reported to be effective in the treatment of dysmenorrhea premature labor and threatened abortion Recent clinical experience with a highly purified and potent relaxin preparation revealed that it is quite effective as a pelvic softener softening the pelvic tissues including the cervix vagina and vulva Thus it acts to insure easier childbirth

d Uterine Relaxing Factor

This non steroidal substance although not yet isolated as a separate entity from human source material seems to be chemically and physiologically related to relaxin and is therefore included here for the sake of completeness The basic research on this material indicates that it is a hormone and it has been tested for its clinical efficacy It has been reported to be effective in the treatment of dysmenorrhea premature labor and threatened and habitual abortion [3]

e Androgens

Evidence pointing to the ovaries as a source of androgens is more than circumstantial Several investigators have reported that the ovaries secrete androgens Furthermore it now appears that testosterone may be a precursor of estradiol 17β The Leydig like cells found in the region of the ovarian hila is presumably the source of ovarian androgens [39] Females excrete in their urine approximately 18 to 33 per cent less androgens than do males [15]

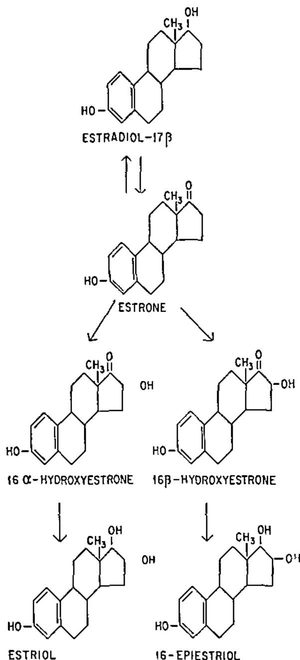


Fig 2 Possible interrelationships of the estrogens determined from human urine (After Marrian G F)

2 The Adenohypophyseal Hormones

As indicated above the adenohypophyseal hormones trigger the ovaries to develop to maturity and se

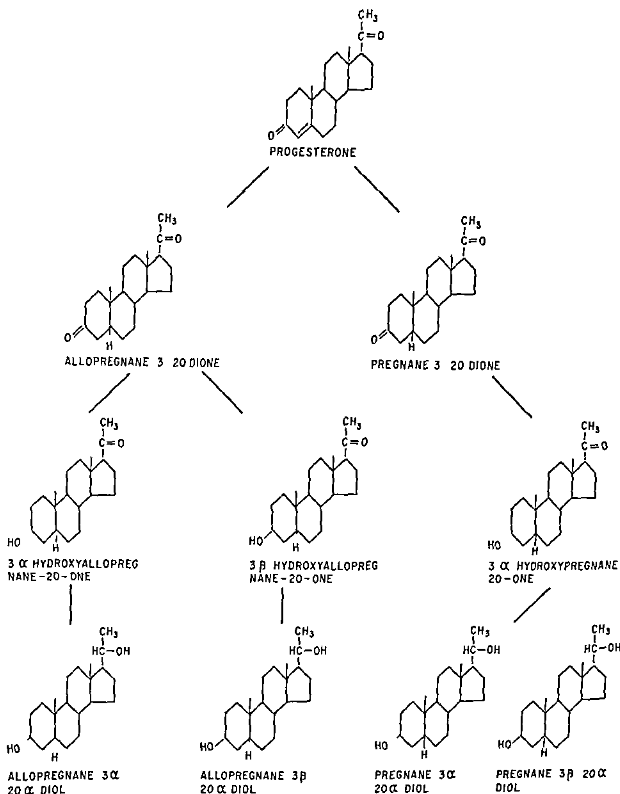


FIG 3 C 21 METABOLITES RELATED TO PROGESTERONE (From Velardo J T *The Endocrinology of Reproduction* Oxford University Press New York)

crete their steroid hormones (estrogenic and progestative substances) During adolescence (usually said to be between the eleventh to sixteenth year of life for the female with a mode around the thirteenth year) a reciprocal adenohypophyseal ovarian relationship is established This relationship exists throughout the normal physiologic career of the reproductive tract from the menarche to the menopause While we do not know the primacy of the adenohypophyseal hormones i e we do not know which of the pituitary gonadotropins appears in the blood stream and acts upon the ovary first experimental evidence from highly controlled and rigidly standardized experiments in laboratory animals suggests the following

a The Follicle Stimulating Hormone (FSH)

FSH is responsible for the following

(1) Growth of ovarian follicles
(2) Maturation of the granulosa cells by causing them to divide and secrete follicular liquor
(3) Further development of the theca interna of the ovarian follicles

b The Luteinizing Hormone (LH)

LH or sometimes referred to as the interstitial cell stimulating hormone ICSH seems essential for

(1) The maturation of the ovarian follicle only after the initial action of FSH
(2) The secretion of estrogenic hormones from the theca interna only after the action of FSH
(3) Ovulation only after the action of FSH
(4) The transformation of the ruptured (ovulated) follicle to the corpus luteum

c Luteotropic Hormone (LtH)

LtH or often referred to as the lactogenic hormone has the chief property of initiating the following

(1) The secretion of progesterone (or progestational substances) from the corpus luteum and
(2) Stimulating the mammary gland after the action of several other required hormones to produce milk after the birth of young

3 The Inter relationships Between Ovarian and Adenohypophyseal Hormones

In laboratory animals it has been shown that the combined actions of FSH and LH are responsible for the secretion of estrogenic hormones from the theca interna After the blood titer of estrogenic hormones reaches a high level it seems that the circulating estrogens act back on the adenohypophysis and restrict the normal release of FSH and simultaneously cause a release of LH Progestational hormones are normally secreted just prior to and after ovulation and when the progestational hormones (progesterone Δ^4 3 ketopregnene 20αol and Δ^4 3 ketopregnene 20βol) reach a level they restrict the normal release of adenohypophyseal LH and simultaneously stimulate the release of FSH It is encouraging to report that a great deal of endocrine research in the human female is taking place Albert and his associates concluded that the effect of estrogen on the excretion of gonadotropins depends on both dosage and the type of estrogen administered [36] Small doses appear to stimulate excretion of gonadotropin This effect was also noted in a young ovariectomized woman in whom the excretion of gonadotropin was presumably maximal Larger dosages of estrogen resulted in the inhibition of urinary gonadotropin Unfortunately no conclusion can be made as to the effect of estrogen on the FSH LH and/or LtH components since their assays measured total urinary gonadotropins In their studies on the effects of progesterone on urinary gonadotropin Smith and Albert [37] concluded that no direct evidence for the various theoretical claims that progesterone regulates the secretion of FSH LH and/or LtH could be obtained from their experiments in the human female

Much less is known of the combined actions of estrogenic and progestational hormones on the adenohypophyseal hormones One report from Hisaw s laboratory indicates that the daily administration of 10 0 μg of estradiol 17β and 2 0 20 0 mg of progesterone to ovariectomized monkeys resulted in a complete loss of adenohypophyseal gonadotropins [33]

As yet we do not have purified adenohypophyseal gonadotropins for use in the human but the recent impetus to obtain human pituitary hormones may soon provide us with a much clearer concept of the adenohypophyseal ovarian inter relationships

B ENDOCRINE RELATIONSHIPS IN THE MALE

The male reproductive system consists of the primary sex organs the testes and the accessory male sexual structures (Fig 4) The testes have two main functions (1) the production of the male sex cells spermatozoa (sperm) and (2) the secretion of the male sex hormone testosterone The male sex hormone is responsible for the growth development and function of the accessory male sex organs and the development of the so called attributes of masculinity beard deep voice and firm musculature The accessory sexual organs consists of the excretory ducts through which the spermatozoa travel from

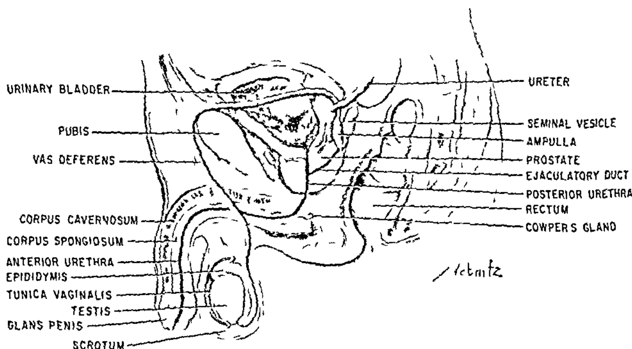


FIG 4 THE MALE GENITAL ORGANS shown in sagittal section (Courtesy of Sohval A R in *The Endocrinology of Reproduction* ed Velardo J T Oxford University Press New York)

the testes to outside the body an associated group of glands (seminal vesicles prostate and bulbo urethral glands) which contribute secretions to the semen and the penis

Ejaculated semen has a viscous creamy slightly yellowish or grayish fluid appearance and consists of two main components spermatozoa and the fluid portion the seminal plasma The composition of semen depends quite largely upon the proportion of sperm and seminal plasma In the adult human male the average volume of a single ejaculate is approximately 2 5 to 3 5 ml The sperm density however is usually less than 100 000 sperm/µl Therefore approximately 10 per cent of the ejaculate is sperm whereas the remaining 90 per cent is seminal plasma [25]

1 The Testicular Hormones

The chief androgen in the human male is testosterone and it is produced by the testis It is fairly well established that the liver converts testosterone into androsterone and etiocholane 3(α) ol 17 one

There are several reports which indicate that the testis of the human secretes an estrogenic substance but most of the data is circumstantial Both the Sertoli and Leydig cells have been suggested as the source of testicular estrogens [3]

There are five principal androgens found in the blood and urine testosterone Δ^4 androstane 3 17 dione 11β hydroxy Δ androstane-3 17 dione dehydroepiandrosterone and adrenosterone The structural formulas and relationships of some androgens non androgenic derivatives and hormones of the ovary and adrenal cortex are shown in Figure 5 The testis produces testosterone the other androgens listed above including Δ^4 androstane 3 17 dione and 11β hydroxyepiandrosterone are produced by the adrenal cortex The metabolic pathway of testosterone is shown in Figure 6 It is interesting to point out here that biologically active androgens are present in the urine of men women and children of both sexes The normal urinary metabolites of the biologically active androgens are androsterone and dehydroepiandrosterone

2 The Adenohypophyseal Hormones

The adenohypophyseal gonadotropins trigger the testes to develop to maturity and to secrete their steroid hormones The period of adolescence heralds the pituitary testicular reciprocal relationship Briefly stated the adenohypophyseal gonadotropins stimulate and regulate the biologic activity of the testis There is rather good evidence for the fact that the testicular androgens act back on the adenohypophysis thus regulating its activity

In the section on the endocrine relationships in the female it was mentioned that the follicle stimulating hormone (FSH) luteinizing hormone (LH)

and luteotropic hormone (LtH) were the adenohypophyseal hormones responsible for ovarian action These same gonadotropins are responsible for testicular activity FSH acts primarily on the epithelium of the seminiferous tubules to stimulate spermatogenesis LH stimulates the interstitial cells (cells of Leydig) to produce androgenic hormones thus far no specific action has been ascribed to LtH in the male The suggestion has been made that LtH stimulates the Leydig cells of the testis to produce androgenic hormones This fact however lacks confirmation

An explanation is in order in regard to the naming of the gonadotropic hormones It has been mentioned that LH stimulates the interstitial cells of Leydig in the testis to produce androgenic hormones Therefore the California group under the direction of H M Evans reserves the right to call this substance the interstitial cell stimulating hormone (ICSH) Furthermore since FSH and LH (ICSH) are terms applied to the effects on the ovary and testis and since these gonadotropins are the same in both sexes the more correct names thylakentrin for FSH and metakentrin for LH have been proposed by Coffin and van Dyke [9] Unfortunately these terms have not gained currency in the literature therefore the terms FSH and LH will probably remain with us until we gain some new and different information on the biologic activity of highly purified and potent gonadotropic preparations

3 The Inter relationships Between Testicular and Adenohypophyseal Hormones

The hormones of the testis exert a feed back action on the adenohypophyseal gonadotropins There is an overwhelming amount of data from animal experiments and clinical observations which give added credence to this established principle Recently Greep and Jones [1] Sohval [3] and Velardo [4] have summarized the literature on this aspect of reproductive endocrinology Suffice it to say that gonadectomy in either sex results in the enlargement of the pituitary gland It has been demonstrated that a correlation exists between increased pituitary weight and augmented gonadotropic function after gonadectomy in laboratory animals Administration of androgens to such animals markedly reduces the amount of adenohypophyseal gonadotropins thus proving

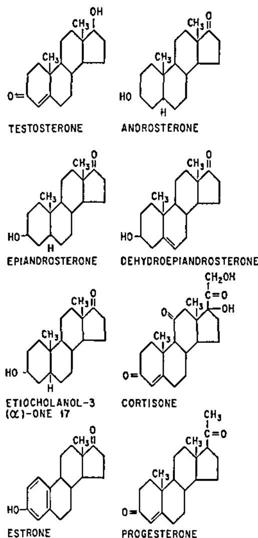


FIG 5 STRUCTURAL FORMULAS AND RELATIONSHIPS OF SOME ANDROGENS non androgenic derivatives and hormones of the ovary and adrenal cortex (Courtesy of Sohval A R in *The Endocrinology of Reproduction* ed Velardo J T Oxford University Press New York)

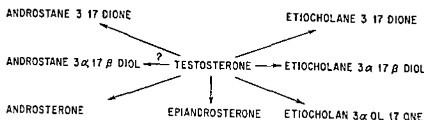


FIG 6 METABOLIC PATHWAY OF TESTOSTERONE (Adapted from Dorfman R I and Shipley R A)

that the amount of circulating androgens influences the pituitary content of gonadotropins Furthermore it is well known that testosterone suppresses the FSH content of the adenohypophysis of the non-orchidectomized or non ovariectomized animal

These pituitary testicular hormonal relationships have received a tremendous amount of confirmation from clinical observations in man Several investigators have shown that an increased gonadotropic titer exists in ovariectomized and orchidectomized human patients Likewise an elevation of urinary gonadotropins has been found in patients with infertility and/or cryptorchidism Finally the administration of large doses of androgens or estrogens has been found to suppress the FSH activity of normal men these gonadal steroids likewise reduce the excessive urinary excretion of pituitary gonadotropins in hypogonadal men and ovariectomized women

4 Origin of Sperm

The male sex cells spermatozoa originate in the testis from the germ (spermatogenic) cells of the seminiferous epithelium during the process of spermatogenesis Two distinct phases are involved in spermatogenesis proliferation and transformation Both of these phases follow a highly organized succession of stages from spermatogonia to primary and secondary spermatocytes spermatids and finally to spermatozoa (Fig 7) Spermatogenesis may occur in the human from puberty (11 18 years generally) until late into the fifth or sixth decade of life In some men it has been known to occur until the ninth and tenth decades of life

Immature spermatozoa are usually always found in close association with Sertoli cells in the testis It is not uncommon to find the head piece of the immature spermatozoon embedded in the cytoplasm of the Sertoli cell Gradually these immature sperm acquire varying degrees of maturity in so doing they become detached from the Sertoli cells and start their migration from the testis to the tubules of the epididymis where they are stored and undergo further maturity Hotchkiss has indicated that sperm are also stored in the ductus deferens [3]

5 Passage and Maturity of Sperm

The passageway taken by the spermatozoa is directly associated with the mechanisms involved in their maturity The actual course of the sperm from the seminiferous tubules to outside the body can be summarized as follows (a) from the seminiferous tubules near the mediastinum of the testis each lobule unites forms a straight tubule enters the mediastinum and opens into the group of large flattened ducts the rete testis (b) from the rete testis the ductuli efferentes (another series of ducts) pass right into the epididymis (c) the ductuli efferentes communicate outside the testis with a common duct the ductus epididymidis (d) the epididymidis courses the entire epididymis in a highly tortuous manner and before emerging from it becomes the ductus deferens (e) leading to the constricted narrower portion the ejaculatory duct and finally (f) to the area between the ductus deferens and its constricted portion to the urethra It is well known that spermatozoa are stored in the vas deferens During ejaculation spermatozoa are released through the passageway of the urethra

Knowledge concerning the chemical changes which take place during spermatogenesis is quite meager with the exception of the several histochemical observations Mann's excellent treatise on the biochemistry of semen summarized and evaluated the relevant data from the several species thus far investigated The following therefore is perforce of a theoretical nature insofar as the human male is concerned (a) spermatogonia and spermatocytes have a basophilic cytoplasm in contrast to the mature spermatozoa which exhibit only a faint trace of basophilia in the flagella of the sperm (b) spermatogenesis involves a progressive disappearance of ribonucleic acid from the developing sperm cell (c) ejaculated spermatozoa of the bull contain 0.2×10^{-9} mg ribonucleic acid per sperm cell or fifteen times less than the corresponding value for desoxyribonucleic acid (estimated analytically according to the 1945 methods of Schmidt and Thannhauser and Schneider) (d) the ribonucleic acid is not formed by the developing germ cells but is secreted by the surrounding cells and then absorbed and utilized by the gametes (e) there is a progressive decline of alkaline and acid phosphatase activity in the nuclei and also a simultaneous disappearance of glycogen (f) the Sertoli cells spermatogonia and to a lesser extent the primary spermatocytes are heavily laden with glycogen (g) secondary spermatocytes and spermatids do not give evidence of any stainable glycogen (h) in the deer and rat sudanophilic material is primarily in the Sertoli cells but in man a heavy deposition of lipid material was found in the Sertoli cells as well as in the cytoplasm of spermatogonia and of primary spermatocytes [5]

The several cytochemical events stated above continue during the storage of spermatozoa in the epididymis Therefore it may be said that certain of these cytochemical expressions are associated with maturity (or ripening) of spermatozoa Very little is known concerning the metabolism of the non motile epididymal spermatozoa

During the process of sperm ripening in the epi

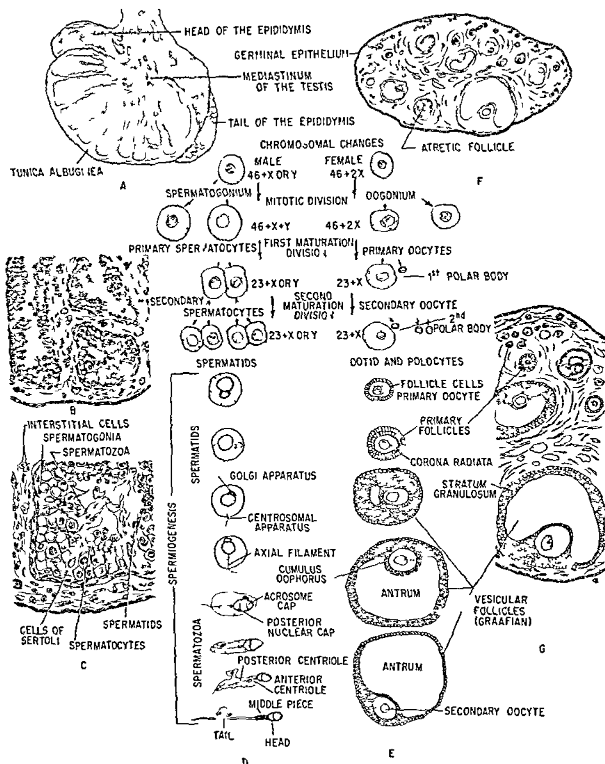


Fig 7 Gametogenesis—the process of spermatogenesis is represented on the left side of the drawing which shows a longitudinal section of the testis at *A* and drawings at increasing magnification at *B* and *C* which demonstrate the seminiferous tubules and interstitial tissues The column of cells at *D* represents diagrammatically the process of spermatogenesis and spermiogenesis Oogenesis is similarly represented at *E* and a longitudinal section of the ovary is represented at *F* and a portion of that section under higher magnification at *G* Although the diploid number of chromosomes is here represented as being 48 the reader is referred to the work of Tjio and Levan (The Chromosome Number of Man Hereditas 42 1 16 1956) which indicates that it may actually be 46 (Courtesy Allan F In *The Endocrinology of Reproduction* ed Velardo J T Oxford University Press New York)

didymis a marked change is noted in the migration of a drop like swelling of the sperm cytoplasm The name kinoplasmic droplet has been given to this lipid material Examination of spermatozoa from the caput epididymidis of the mouse reveals that the kinoplasmic droplet is usually found in the proximal end of the middle piece This droplet is usually found in the distal end of the middle piece when the sperm reach the cauda epididymidis near the vas deferens In ejaculated sperm however these droplets are infrequently found except in cases of infertility The *migration and disappearance of the kinoplasmic* droplet is seemingly the final stage in the process of gradual shrinkage and dehydration of protoplasm associated with spermatogenesis and sperm maturity

Mann's monograph on the biochemistry of semen also indicated that the epididymis is adapted for long term storage of spermatozoa more specifically some secretion from the cells of the ductus epididymidis and the changing cytochemical events or the fructose therein contained seem to be important factors in the maturation of spermatozoa [2]

6 Additional Data on Human Spermatozoa

In addition to the pieces of information above concerning spermatozoa there are some chemical and physical characteristics available for human sperm and/or semen It has been reported that human sperm (or semen if so indicated) possess (a) an electrical charge measured at 20 C 88 107 reciprocal ohms $\times 10^{-4}$ (b) an osmotic pressure in terms of freezing point depression 0 55 0 58 C (c) *hydrogen ion concentration (of semen)* 7 35 to 7 50 (d) average specific gravity (whole semen) of 1 028 (e) adenosine triphosphate (ATP) representing an intracellular constituent and an important co enzyme in the over all metabolic economy of spermatozoa (f) trace amounts of ergothioneine (g) citric acid (479 mg /100 ml semen) (h) protein constituents and enzymes (in semen albumins globulins nucleoproteins spermine protease mucoprotein hexoseamine plus a large number of substances not yet unequivocally established in sperm *hyaluronidase)* [25]

C FERTILIZATION

Fertilization is the successful union of the ovum with a spermatozoon and usually occurs after the ovum enters the fimbriated end of the oviduct (Fallopian tube) *It is not known how long the human ovum* maintains its physiological competence to be fertilized but it has been suggested that it is not in excess of 24 hours The fertilized ovum utilizes about 4 days to traverse the uterine tube and implantation usually occurs 2 3 days after the arrival of the fertilized ovum in the uterus

Recent evidence indicates that the process of sperm ripening does not necesarily stop at ejaculation but continues in the female reproductive tract after copulation It is believed that the sperm cell undergoes definite changes—called capacitation—before it is truly competent physiologically to penetrate the surface of an available ovum

Therefore it seems quite likely that the success achieved in fertilization with epididymal spermatozoa *may well be due to progressive ripening of the sperm* in the female reproductive tract

Experiments in the cow sow and rabbit indicate that a definite period of time is required for the travel of adequate numbers of sperm to the oviducts for fertilization irrespective of the placing of ejaculated sperm in the uterus cervix or vagina The time required for sperm to arrive at the site of fertilization varies for each species 15 minutes in the rat 2 5 minutes in the rabbit and from the best estimates at hand anywhere from 6 to 24 hours in the human female

Although the sperm reach the site of fertilization *in a given period of time it should not be interpreted* to mean that this is the actual time required for fertilization Greenwald has reported that within 3 to 4 hours after copulation half of the liberated ova in the oviduct of the rabbit were fertilized but at 5 hours all of the available ova were fertilized [18]

In his very scholarly treatise and critical evaluation of *How Do Sperms Get into the Uterus?* Dr Carl G Hartman [19] has performed a commendable service in that he has truly informed us as to the status of this very important aspect of reproductive physiology From his survey of 153 scientific papers covering a period of 88 years Dr Hartman concludes that there are indeed several possibilities—or combinations thereof—as regards the mechanisms of sperm entry from the vagina into the uterus There are numerous obstacles confronting the migration of spermatozoa by their own efforts therefore it seems that nature has provided a more certain method specifically the co operation of the musculature of the female reproductive tract Dr Hartman is of the opinion that there is an *en masse* entry of spermatozoa from the vagina into the cervix and that sperm motility *per se* is the means whereby they travel through the oviduct except perhaps in the passage of the second hurdle the utero tubal junction Therefore from these facts it seems that the chief function *of the flagellum of the spermatozoan is the pene*tration of the corona radiata the zona pellucida and the vitelline membrane of the liberated ovum a conclusion consistent with Dr Hartman's thoughts as expressed in his critical survey and the photographic

documentation of the fertilization process by Blandau [8] and the several investigations by Austin [2,4]

D NATURE OF HORMONAL ACTIONS IN REPRODUCTIVE MECHANISMS

Recently it has become more apparent than real that the reproductive tract is influenced by the sum total of all the hormones and their metabolites in a physiological system at a specific time [40,42,48] Numerous experiments in laboratory animals have provided both a theoretical explanation of (1) certain gynecologic dysfunctions and (2) endocrine action *in reproductive mechanisms* While these data are not fully capable of being translated to the human female they do shed light on areas of human reproduction that warrant closer study

Hisaw [20] and Velardo [45] and their collaborators have shown that uterine growth in rats is influenced by the action of the estrogens *in concert* and that the uterine growth promoting action of both estradiol 17β and estrone can be markedly restricted by estriol and 16 epi estriol Moreover there are interactions among progesterone and its C 21 related metabolites It has been shown that the C 21 related metabolites of progesterone can interfere with the action of progesterone in decidual tissue formation in rats More recently it was reported that there are competitive interactions among hormones of the adrenal cortex and the ovaries (estrogens and progestins) A detailed review of these numerous actions and interactions is included in a recent survey by Velardo [42]

It is well known that there are numerous actions and interactions among hormones of the adenohypophysis and the gonads There is additional evidence indicating that a thyroid hormone triiodothyronine can modify the ratios of the metabolites of testosterone This further emphasizes the fact that one must be concerned with the physiologic actions of other glands besides those of the reproductive system if one aims to unravel the numerous complexities of reproductive endocrinology It should be emphasized that we are most interested in the interactions between *or among* the thyroidal and gonadal hormones [44] especially since it has been reported that testosterone is a precursor of the naturally occurring *female sex hormones* [6]

Mid twentieth century research is moving rapidly into the intricate area of enzymes and hormones [9] Giving added impetus to this are the recent conferences on the influence of hormones on enzymes The future will undoubtedly clarify the exacting relationships among specific enzymes and hormones on the several metabolic alterations of the reproductive tract

E HORMONE LEVELS IN THE HUMAN MALE AND FEMALE

1 Hormones in the Female

a GONADOTROPINS

The best available and most complete data on the gonadotropic hormone levels of the human have been reported from Albert s laboratory (Tables 1 and 2) [1]

Albert and his colleagues were successful in determining the urinary gonadotropin values (combined gonadotropins) of 4 healthy women during several menstrual cycles These assays based on 92 determinations reveal that the higher values occur near the mid cycle These findings are in essential agreement with the so-called ovulatory rise of gonadotropins observed by others

It may prove of interest to examine the values of urinary gonadotropins as a function of age These

Table 1 COMPARISON OF GONADOTROPIC ACTIVITY OF THE PITUITARY GLAND AT VARIOUS AGES

Source	Approximate weight of pituitary mg	Total amount units per pituitary		Ratio FSH/LH
		FSH	LH	
Infant	70	0	0	—
Child	200	>6	<6	>1
Adult Man	300	100	100	1
Adult Woman	600	200	200	1
Postmenopausal woman	600	600	200	3

Courtesy of Albert A et al Hormones and the Aging Process Academic Press N Y 1956

Table 2 COMPARISON OF GONADOTROPIC ACTIVITY OF PITUITARY TISSUE AND OF URINE OF YOUNG ADULT MEN AND WOMEN

Source	Units per kg		Units per gland or per day		Ratio FSH/LH
	FSH	LH	FSH	LH	
Pituitary	330 000	330 000	150	150	1
Urine	2	<1	2	<1	2

Courtesy of Albert A et al Hormones and the Aging Process Academic Press N Y 1956

didymis a marked change is noted in the migration of a drop like swelling of the sperm cytoplasm The name kinoplasmic droplet has been given to this lipid material Examination of spermatozoa from the caput epididymidis of the mouse reveals that the kinoplasmic droplet is usually found in the proximal end of the middle piece This droplet is usually found in the distal end of the middle piece when the sperm reach the cauda epididymidis near the vas deferens In ejaculated sperm however these droplets are infrequently found *except in cases of infertility* The migration and disappearance of the kinoplasmic droplet is seemingly the final stage in the process of gradual shrinkage and dehydration of protoplasm associated with spermatogenesis and sperm maturity

Mann s monograph on the biochemistry of semen also indicated that the epididymis is adapted for long term storage of spermatozoa more specifically some secretion from the cells of the ductus epididymidis and the changing cytochemical events or the *fructose therein contained seem to be important* factors in the maturation of spermatozoa[2]

6 Additional Data on Human Spermatozoa

In addition to the pieces of information above concerning spermatozoa there are some chemical and physical characteristics available for human sperm and/or semen It has been reported that human sperm (or semen if so indicated) possess (a) an electrical charge measured at *20 C 88 107* reciprocal ohms $\times 10^{-4}$ (b) an osmotic pressure in terms of freezing point depression 0 55 0 58 C (c) hydrogen ion concentration (of semen) 7 35 to 7 50 (d) average specific gravity (whole semen) of 1 028 (e) adenosine triphosphate (ATP) representing an intracellular constituent and an important co enzyme in the over all metabolic economy of spermatozoa (f) trace amounts of ergothioneine (g) citric acid (479 mg /100 ml semen) (h) protein constituents and enzymes (in semen albumins globulins nucleoproteins spermine protease mucoprotein hexoseamine plus a large number of substances not yet unequivocally established in sperm hyaluronidase)[25]

C FERTILIZATION

Fertilization is the successful union of the ovum with a spermatozoon and usually occurs after the ovum enters *the fimbriated* end of the oviduct (Fallopian tube) It is not known how long the human ovum maintains its physiological competence to be fertilized but it has been suggested that it is not in excess of 24 hours The fertilized ovum utilizes about 4 days to traverse the uterine tube *and implantation* usually occurs 2 3 days after the arrival of the fertilized ovum in the uterus

Recent evidence indicates that the process of sperm ripening does not necesarily stop at ejaculation but continues in the female reproductive tract after copulation *It is believed that the sperm cell* undergoes definite changes—called capacitation—before it is truly competent physiologically to penetrate the surface of an available ovum

Therefore it seems quite likely that the success *achieved in fertilization with epididymal spermatozoa* may well be due to progressive ripening of the sperm in the female reproductive tract

Experiments in the cow sow and rabbit indicate that a definite period of time is required for the *travel of adequate numbers of sperm to the oviducts* for fertilization irrespective of the placing of ejaculated sperm in the uterus cervix or vagina The time required for sperm to arrive at the site of fertilization varies for each species 15 minutes in the *rat 2 5 minutes in the rabbit and from the best* estimates at hand anywhere from 6 to 24 hours in the human female

Although the sperm reach the site of fertilization in a given period of time it should not be interpreted *to mean that this is the actual time required for* fertilization Greenwald has reported that within 3 to 4 hours after copulation half of the liberated ova in the oviduct of the rabbit were fertilized but at 5 hours all of the available ova were fertilized[18]

In his very scholarly treatise and critical evaluation of How Do Sperms Get into the Uterus? Dr Carl G Hartman[19] has performed a commendable service in that he has truly informed us as to the status of this very important aspect of reproductive *physiology From his survey of 153 scientific papers* covering a period of 88 years Dr Hartman concludes that there are indeed several possibilities—or combinations thereof—as regards the mechanisms of sperm entry from the vagina into the uterus There *are numerous obstacles confronting the migration of* spermatozoa by their own efforts therefore it seems that nature has provided a more certain method specifically the co operation of the musculature of the female reproductive tract Dr Hartman is of the *opinion that there is an en masse* entry of spermatozoa from the vagina into the cervix and that sperm motility *per se* is the means whereby they travel through the oviduct except perhaps in the passage of the second hurdle the utero tubal junction Therefore from these facts it seems that the chief function of the flagellum of the spermatozoan is the penetration of the corona radiata the zona pellucida and the vitelline membrane of the liberated ovum a conclusion consistent with Dr Hartman s thoughts as *expressed in his critical survey* and the photographic

documentation of the fertilization process by Blandau[8] and the several investigations by Austin[2,3]

D NATURE OF HORMONAL ACTIONS IN REPRODUCTIVE MECHANISMS

Recently it has become more apparent than real that the reproductive tract is influenced by the sum total of all the hormones and their metabolites in a physiological system at a specific time[40,41,44,8] Numerous experiments in laboratory animals have provided both a theoretical explanation of (1) certain gynecologic dysfunctions and (2) endocrine action in reproductive mechanisms While these data are not fully capable of being translated to the human female they do shed light on areas of human reproduction that warrant closer study

Hisaw[20] and Velardo[43] and their collaborators have shown that uterine growth in rats is influenced by the action of the estrogens *in concert* and that the uterine growth promoting action of both estradiol 17β and estrone can be markedly restricted by estriol and 16 epi estriol Moreover there are interactions among progesterone and its C 21 related metabolites It has been shown that the C 21 related metabolites of progesterone can interfere with the action of progesterone in decidual tissue formation in rats[1] More recently it was reported that there are competitive interactions among hormones of the adrenal cortex and the ovaries (estrogens and progestins) A detailed review of these numerous actions and interactions is included in a recent survey by Velardo[42]

It is well known that there are numerous actions and interactions among hormones of the adenohypophysis and the gonads There is additional evidence indicating that a thyroid hormone triiodothyronine can modify the ratios of the metabolites of testosterone This further emphasizes the fact that one must be concerned with the physiologic actions of other glands besides those of the reproductive system if one aims to unravel the numerous complexities of reproductive endocrinology It should be emphasized that we are most interested in the interactions between or among the thyroidal and gonadal hormones[14] especially since it has been reported that testosterone is a precursor of the naturally occurring female sex hormones

Mid twentieth century research is moving rapidly into the intricate area of enzymes and hormones[1] Giving added impetus to this are the recent conferences on the influence of hormones on enzymes The future will undoubtedly clarify the exacting relationships among specific enzymes and hormones on the several metabolic alterations of the reproductive tract

E HORMONE LEVELS IN THE HUMAN MALE AND FEMALE

1 Hormones in the Female

a GONADOTROPINS

The best available and most complete data on the gonadotropic hormone levels of the human have been reported from Albert s laboratory (Tables 1 and 2)

Albert and his colleagues were successful in determining the urinary gonadotropin values (combined gonadotropins) of 4 healthy women during several menstrual cycles These assays based on 92 determinations reveal that the higher values occur near the mid cycle These findings are in essential agreement with the so called ovulatory rise of gonadotropins observed by others

It may prove of interest to examine the values of urinary gonadotropins as a function of age These

Table 1 COMPARISON OF GONADOTROPIC ACTIVITY OF THE PITUITARY GLAND AT VARIOUS AGES

Source	Approximate weight of pituitary mg	Total amount units per pituitary		Ratio FSH/LH
		FSH	LH	
Infant	70	0	0	—
Child	200	>6	<6	>1
Adult Man	300	100	100	1
Adult Woman	600	200	200	1
Postmenopausal woman	600	600	200	3

Courtesy of Albert A et al Hormones and the Aging Process Academic Press N Y 1956

Table 2 COMPARISON OF GONADOTROPIC ACTIVITY OF PITUITARY TISSUE AND OF URINE OF YOUNG ADULT MEN AND WOMEN

Source	Units per kg		Units per gland or per day		Ratio FSH/LH
	FSH	LH	FSH	LH	
Pituitary	330 000	330 000	150	150	1
Urine	2	<1	2	<1	2

Courtesy of Albert A et al Hormones and the Aging Process Academic Press N Y 1956

didymis a marked change is noted in the migration of a drop like swelling of the sperm cytoplasm The name kinoplasmic droplet has been given to this lipid material Examination of spermatozoa from the caput epididymidis of the mouse reveals that the kinoplasmic droplet is usually found in the proximal end of the middle piece This droplet is usually found in the distal end of the middle piece when the sperm reach the cauda epididymidis near the vas deferens In ejaculated sperm however these droplets are infrequently found except in cases of infertility The migration and disappearance of the kinoplasmic droplet is seemingly the final stage in the process of gradual shrinkage and dehydration of protoplasm associated with spermatogenesis and sperm maturity

Mann s monograph on the biochemistry of semen also indicated that the epididymis is adapted for long term storage of spermatozoa more specifically some secretion from the cells of the ductus epididymidis and the changing cytochemical events or the fructose therein contained seem to be important factors in the maturation of spermatozoa

6 Additional Data on Human Spermatozoa

In addition to the pieces of information above concerning spermatozoa there are some chemical and physical characteristics available for human sperm and/or semen It has been reported that human sperm (or semen if so indicated) possess (a) an electrical charge measured at 20 C 88 107 reciprocal ohms $\times$ 10 ^{4} (b) an osmotic pressure in terms of freezing point depression 0 55 0 58 C (c) hydrogen ion concentration (of semen) 7 35 to 7 50 (d) average specific gravity (whole semen) of 1 028 (e) adenosine triphosphate (ATP) representing an intracellular constituent and an important co enzyme in the over all metabolic economy of spermatozoa (f) trace amounts of ergothioneine (g) citric acid (479 mg/100 ml semen) (h) protein constituents and enzymes (in semen albumins globulins nucleoproteins spermine protease mucoprotein hexoseamine plus a large number of substances not yet unequivocally established in sperm hyaluronidase) [2]

C FERTILIZATION

Fertilization is the successful union of the ovum with a spermatozoon and usually occurs after the ovum enters the fimbriated end of the oviduct (Fallopian tube) It is not known how long the human ovum maintains its physiological competence to be fertilized but it has been suggested that it is not in excess of 24 hours The fertilized ovum utilizes about 4 days to traverse the uterine tube and implantation usually occurs 2 3 days after the arrival of the fertilized ovum in the uterus

Recent evidence indicates that the process of sperm ripening does not necesarily stop at ejaculation but continues in the female reproductive tract after copulation It is believed that the sperm cell undergoes definite changes—called capacitation—before it is truly competent physiologically to penetrate the surface of an available ovum

Therefore it seems quite likely that the success achieved in fertilization with epididymal spermatozoa may well be due to progressive ripening of the sperm in the female reproductive tract

Experiments in the cow sow and rabbit indicate that a definite period of time is required for the travel of adequate numbers of sperm to the oviducts for fertilization irrespective of the placing of ejaculated sperm in the uterus cervix or vagina The time required for sperm to arrive at the site of fertilization varies for each species 15 minutes in the rat 2 5 minutes in the rabbit and from the best estimates at hand anywhere from 6 to 24 hours in the human female

Although the sperm reach the site of fertilization in a given period of time it should not be interpreted to mean that this is the actual time required for fertilization Greenwald has reported that within 3 to 4 hours after copulation half of the liberated ova in the oviduct of the rabbit were fertilized but at 5 hours all of the available ova were fertilized [18]

In his very scholarly treatise and critical evaluation of How Do Sperms Get into the Uterus? Dr Carl G Hartman [19] has performed a commendable service in that he has truly informed us as to the status of this very important aspect of reproductive physiology From his survey of 153 scientific papers covering a period of 88 years Dr Hartman concludes that there are indeed several possibilities—or combinations thereof—as regards the mechanisms of sperm entry from the vagina into the uterus There are numerous obstacles confronting the migration of spermatozoa by their own efforts therefore it seems that nature has provided a more certain method specifically the co operation of the musculature of the female reproductive tract Dr Hartman is of the opinion that there is an *en masse* entry of spermatozoa from the vagina into the cervix and that sperm motility *per se* is the means whereby they travel through the oviduct except perhaps in the passage of the second hurdle the utero tubal junction Therefore from these facts it seems that the chief function of the flagellum of the spermatozoan is the penetration of the corona radiata the zona pellucida and the vitelline membrane of the liberated ovum a conclusion consistent with Dr Hartman s thoughts as expressed in his critical survey and the photographic

any correlation with the stage of the menstrual cycle There are no quantitative values for blood androgens

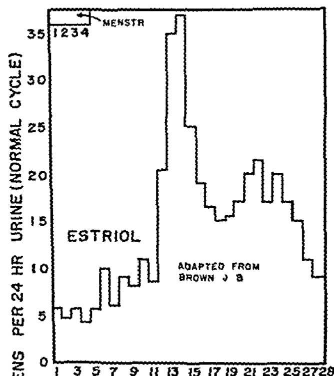
Females excrete in their urine approximately 33 per cent less neutral 17 ketosteroids than do men 5 1 14 2 mg and 8 1 22 6 mg /24 hours for women and men respectively Likewise these substances do not seem to undergo any cyclic change during the menstrual cycle

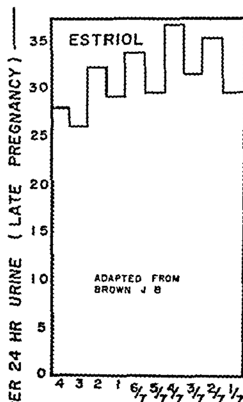
The average daily urinary output of 17 keto steroids androgens and estrogens by normal children and young adults is summarized in Table 3 [34 33]

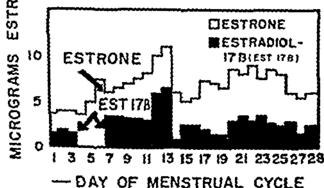
2 Hormones in the Male

a Gonadotropins

Albert and his associates report that there are approximately 5 10 units/24 hours of urinary gonado-







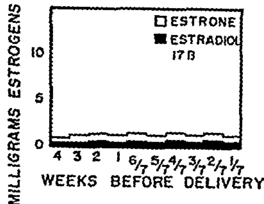


Fig 8 (*left*) Urinary values of estradiol—17 β estrone and estriol during the typical 28-day menstrual cycle of the human female (After Brown J B Conf Society for Endo and Royal Soc Med London 1954 and Brown J B *The Lancet* (1956) I (XX) 704 707)

Fig 9 (*right*) Urinary values of estradiol—17 β estrone and estriol during the last four weeks of pregnancy These are the averages of four human females Note that these averages are in milligrams and are based on 24 hour urine samples (After Brown J B Conf Society for Endo and Royal Soc Med London 1954 and Brown J B *The Lancet* (1956) I (XX) 704 707)

results reveal that the gonadotropins can be detected in the female without difficulty from the fifteenth through the eighty fifth year of life A peak excretion value is usually obtained between the fifty fifth to the seventieth year This same report indicates that there is a fourfold increase from a mean of 5 units in the decade 10 19 years to 20 units in the decade 40 49 In examining the post menopausal values a peak value of urinary gonadotropin is observed between the ages of 50 and 70 in the female In summary then it can be stated that in premenopausal women there appears to be a progressive and a fourfold rise in urinary gonadotropins between the ages of 10 and 50 years After the menopause there is yet a further increase in urinary gonadotropin reflected in a peak value 15 19 years after the menopause This peak value however falls steadily throughout the next two decades [1]

b ESTROGENS

According to the composite curve obtained by Markee and Berg [6] from assaying the bloods of 75 normally menstruating women there are only 20 International Units (I U) of estrogen per liter of blood on the first day of the menstrual cycle a slight rise to 30 I U /l blood from the second to the ninth day of the cycle and a steep rise to 58 I U /l blood from the tenth to the fourteenth day A slight decrease to 50 I U seems to prevail from the fifteenth to the eighteenth day and a slight increase to approximately 54 I U is observed during the nineteenth through the twenty first day of the menstrual cycle The blood level of estrogen returns to the pre ovulatory rise of approximately 26 I U during the remaining portion of the cycle the twenty second to the twenty seventh day of the so called normal 28 day cycle

Thus far it has been extremely difficult to fractionate the individual estrogens from blood therefore the values are given in terms of I U of estrogen The measurements are somewhat more revealing in the urinary assays of the estrogens It is possible to measure estradiol estrone estriol and to a lesser degree—perhaps only qualitatively—16 epi estriol 16α hydroxyestrone and 16β hydroxyestrone from urine collections Most of our knowledge of the quantification of urinary estrogens however are based on estradiol 17β estrone and estriol The curves obtained by the Smiths [35] reveal that the urinary titers of the estrogens parallel those obtained from blood samples More specifically there are only trace values of estradiol 17β a peak for estrone representing almost one third of the urinary estrogen and a large amount of estriol or practically two thirds of the urinary estrogens Recently J B Brown has used a more sensitive method for the detection and quantitation of estradiol—17β estrone and estriol during the typical 28 day menstrual cycle (Fig 8) and pregnancy (Fig 9) His data emphasize the quantitative relationships of these three estrogens in a more exacting manner

c PROGESTERONE (PROGESTINS GESTAGENS)

Forbes reports that free progesterone appears in the blood 24 48 hours before or coincidental with a prominent rise in waking temperature or a few hours before intermenstrual pain (*Mittelschmerz*) The progesterone (progestin) level was observed in two peaks of 1 7 to 5 2 μg /cc plasma after which a decline followed The peak levels were detected on the sixth to the ninth day before the onset of menstruation or approximately on the nineteenth to the twenty second day of a 28 day menstrual cycle The hormonal substance(s) did not disappear until after the onset of menses It is interesting to note that the rise and fall of progestins and waking temperatures in each of the menstrual cycles studied paralleled one another Bound progestin levels were consistently low and undetectable [12] These data seem to indicate that there is considerably more progestin than estrogen secreted by the human ovaries during the menstrual cycle

There are at least 10 urinary metabolites of progesterone (progestins) pregnanediols pregnanolones pregnanediones and probably other C-21 related metabolites The pregnanediols and pregnanolones are excreted in both the 3 alpha and 3 beta forms These of course are excreted in the urine as glucuronides It is worth repeating that since pregnanediol is a metabolite of progesterone it is often measured clinically and interpreted as an index of progesterone secretion by the corpus luteum Pregnanediol or its glucuronide sodium pregnanediol glucuronide (NaPG) is almost always absent from the urine during the greater part of the proliferative phase of the menstrual cycle Current evidence indicates that it rises steadily after ovulation reaches a maximum during the peak aspect of luteal activity and then falls to very low or undetectable levels just prior to menstruation The average total amount of pregnanediol excreted during a normal luteal phase has been variously estimated between 30 and 60 mg with an average close to 50 mg

d ANDROGENS AND NEUTRAL 17 KETOSTEROIDS

Females excrete in their urine approximately 25 per cent less androgens than do males Gallagher and his associates reported that females excrete 42 56 I U androgens/24 hours in their urine whereas it was found that men excrete 63 68 I U daily The daily output of urinary androgens does not show

any correlation with the stage of the menstrual cycle *There are no quantitative values for blood androgens*

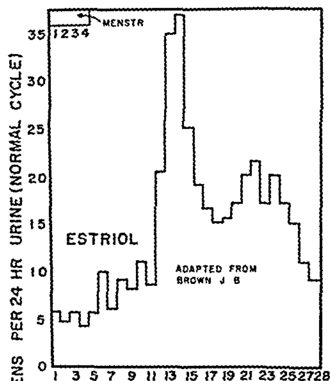
Females excrete in their urine approximately 33 per cent less neutral 17 ketosteroids than do men 5 1 14 2 mg and 8 1 22 6 mg /24 hours for women and men respectively Likewise these substances do not seem to undergo any cyclic change during the menstrual cycle

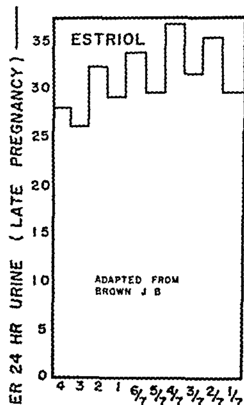
The average daily urinary output of 17 keto-steroids androgens and estrogens by normal children and young adults *is summarized in Table 3*[24,25]

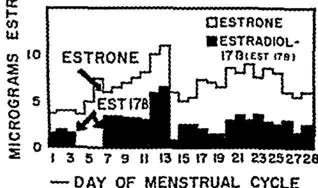
2 Hormones in the Male

a Gonadotropins

Albert and his associates report that there are approximately 5 10 units/24 hours of urinary gonado-







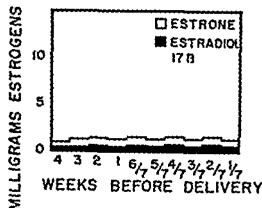


Fig 8 (*left*) Urinary values of estradiol—17 β estrone and estriol during the typical 28-day menstrual cycle of the human female (After Brown J B Conf Society for Endo and Royal Soc Med London 1954 and Brown J B *The Lancet* (1956) I (XX) 704 707)

Fig 9 (*right*) Urinary values of estradiol—17 β estrone and estriol during the last four weeks of pregnancy These are the averages of four human females Note that these averages are in milligrams and are based on 24 hour urine samples (After Brown J B *Conf Society for Endo* and Royal Soc Med London 1954 and Brown J B *The Lancet* (1956) I (XX) 704 707)

results reveal that the gonadotropins can be detected in the female without difficulty from the fifteenth through the eighty fifth year of life A peak excretion value is usually obtained between the fifty fifth to the seventieth year This same report indicates that there is a fourfold increase from a mean of 5 units in the decade 10 19 years to 20 units in the decade 40 49 In examining the post menopausal values a peak value of urinary gonadotropin is observed between the ages of 50 and 70 in the female In summary then it can be stated that in premenopausal women there appears to be a progressive and a fourfold rise in urinary gonadotropins between the ages of 10 and 50 years After the menopause there is yet a further increase in urinary gonadotropin reflected in a peak value 15 19 years after the menopause This peak value however falls steadily throughout the next two decades [1]

b Estrogens

According to the composite curve obtained by Markee and Berg [8] from assaying the bloods of 75 normally menstruating women there are only 20 International Units (I U) of estrogen per liter of blood on the first day of the menstrual cycle a slight rise to 30 I U /l blood from the second to the ninth day of the cycle and a steep rise to 58 I U /l blood from the tenth to the fourteenth day A slight decrease to 50 I U seems to prevail from the fifteenth to the eighteenth day and a slight increase to approximately 54 I U is observed during the nineteenth through the twenty first day of the menstrual cycle The blood level of estrogen returns to the pre ovulatory rise of approximately 26 I U during the remaining portion of the cycle the twenty second to the twenty seventh day of the so called normal 28 day cycle

Thus far it has been extremely difficult to fractionate the individual estrogens from blood therefore the values are given in terms of I U of estrogen The measurements are somewhat more revealing in the urinary assays of the estrogens It is possible to measure estradiol estrone estriol and to a lesser degree—perhaps only qualitatively—16 epi estriol 16α hydroxyestrone and 16β hydroxyestrone from urine collections Most of our knowledge of the quantification of urinary estrogens however are based on estradiol 17β estrone and estriol The curves obtained by the Smiths [35] reveal that the urinary titers of the estrogens parallel those obtained from blood samples More specifically there are only trace values of estradiol 17β a peak for estrone representing almost one third of the urinary estrogen and a large amount of estriol or practically two-thirds of the urinary estrogens Recently J B Brown has used a more sensitive method for the detection and quantitation of estradiol—17β estrone and estriol during the typical 28 day menstrual cycle (Fig 8) and pregnancy (Fig 9) His data emphasize the quantitative relationships of these three estrogens in a more exacting manner

c Progesterone (Progestins Gestagens)

Forbes reports that free progesterone appears in the blood 24 48 hours before or coincidental with a prominent rise in waking temperature or a few hours before intermenstrual pain (*Mittelschmerz*) The progesterone (progestin) level was observed in two peaks of 1 7 to 5 2 μg/cc plasma after which a decline followed The peak levels were detected on the sixth to the ninth day before the onset of menstruation or approximately on the nineteenth to the twenty second day of a 28 day menstrual cycle The hormonal substance(s) did not disappear until after the onset of menses It is interesting to note that the rise and fall of progestins and waking temperatures in each of the menstrual cycles studied paralleled one another Bound progestin levels were consistently low and undetectable [1] These data seem to indicate that there is considerably more progestin than estrogen secreted by the human ovaries during the menstrual cycle

There are at least 10 urinary metabolites of progesterone (progestins) pregnanediols pregnanolones pregnanediones and probably other C 21 related metabolites The pregnanediols and pregnanolones are excreted in both the 3 alpha and 3 beta forms These of course are excreted in the urine as glucuronides It is worth repeating that since pregnanediol is a metabolite of progesterone it is often measured clinically and interpreted as an index of progesterone secretion by the corpus luteum Pregnanediol or its glucuronide sodium pregnanediol glucuronide (NaPG) is almost always absent from the urine during the greater part of the proliferative phase of the menstrual cycle Current evidence indicates that it rises steadily after ovulation reaches a maximum during the peak aspect of luteal activity and then falls to very low or undetectable levels just prior to menstruation The average total amount of pregnanediol excreted during a normal luteal phase has been variously estimated between 30 and 60 mg with an average close to 50 mg

d Androgens and Neutral 17 Ketosteroids

Females excrete in their urine approximately 25 per cent less androgens than do males Gallagher and his associates reported that females excrete 42 56 I U androgens/24 hours in their urine whereas it was found that men excrete 63 68 I U daily The daily output of urinary androgens does not show

of sexual maturity and yet another rise to 11 0 mg daily is detectable throughout the adult life span of the male (Table 3) [34 38]

d ESTROGENS

Urinary estrogens are found throughout the life span of the human male The urinary estrogens rise steadily from approximately 10 I U daily during infancy to 20 30 I U during the ages 10 11 years These values rise to 30 95 I U during adolescence and fluctuate anywhere from 10 to 100 I U in the adult male (Table 3) [34 38]

F PHYSIOLOGY OF THE SEX HORMONES

1 In Females

a ESTROGENS

Estrogens regulate the growth and development of the female reproductive tract and the mammary glands Metabolic changes are also regulated in part or in whole by estrogens These include a large array of biochemical substances associated with the total body composition (mainly extra gonadal) e g bone growth retention of sodium nitrogen and phosphate in patients with ovarian insufficiency retention of calcium phosphate and nitrogen in post menopausal women with osteoporosis [13] estrogens also exert an effect on acne vulgaris blood volume Krebs s cycle liver cholinesterase protein metabolism capillaries of the skin and other factors too numerous to mention many of which have not been established in the human

The ovaries are stimulated by small doses of naturally occurring and synthetically manufactured estrogens Large amounts exert a definite inhibitory action directly (on the ovaries) and indirectly (by modifying the adenohypophyseal gonadotropins) From laboratory animals and limited urinary analyses of human females given estrogens it has been theorized that e trogens effect a release of pituitary LH and seem important in the ovulatory process The reader is referred to Chapter II for a detailed discussion of ovulation

The *uterine tubes* (oviducts Fallopian tubes) have been reported to undergo cyclic activity with the fluctuating titers of estrogens Both the tubular epithelium and the musculature are directly stimulated by estrogens Recent evidence indicates that the cyclic changes in motility are under the direct influence of estrogenic and progestational hormones

The endometrium (uterine mucosa) undergoes cyclic changes in growth and development in response to estrogens The glandular epithelium and the epithelial cells lining the surface of the endometrium are stimulated to proliferate during the estrogenic phase (follicular first half of the menstrual cycle) Also affected by the circulating estrogens are the spiral arterioles and the stromal components of the uterine mucosa It has been demonstrated and confirmed universally that estrogen administered to the ovariectomized monkey and human female induces rapid growth of the endometrium increased vascularity glandular proliferation and most conspicuously numerous mitoses in the glandular and surface epithelium and stromal edema

Uterine contractions and myometrial growth are also stimulated by the estrogenic hormones Current opinion suggests that the estrogens stimulate the quantity and the quality of the contractions This area of human reproduction is undergoing a great deal of careful scrutiny and the current researches of Csapo S R M Reynolds and R Caldevro Barcia will undoubtedly clarify our concepts of uterine contractions that occur during the non gravid and gravid states

The cervix likewise responds to the growth promoting action of the estrogens The glands of the cervix during the preovulatory (estrogenic) phase contain a greater amount of alkaline mucus with a lowered viscosity This condition seems to favor sperm migration motility and longevity

The vaginal epithelium undergoes rapid proliferation and marked growth (numerous mitoses and an increase in the number of cellular layers) with the initial appearance of the ovarian estrogens Normally estrogens induce proliferation of the vaginal epithelium during the follicular phase

The mammary glands particularly the ducts hypertrophy as a result of estrogenic action To a lesser degree in women the estrogens may induce slight to moderate amount of growth of the lobular alveolar system Complete growth development *and* function of the mammary glands requires estrogen progesterone and a number of pituitary factors

b PROGESTERONE (PROGESTINS GESTAGENS)

It should be emphasized that while estrogen is the chief sex (steroid) hormone secreted by the ovary during the follicular phase prior to the stage known as preovulatory swelling both estrogen and progesterone can be detected in the blood stream of the human female just prior to ovulation and during the functional capacity of the corpus luteum Thus circumstantial evidence points to the fact that progesterone is secreted prior to ovulation and the formation of the corpus luteum Consequently in examining the primacy of the ovarian steroid hormones (estrogens and progestational substances) it appears

Table 3 AVERAGE DAILY URINARY OUTPUT OF 17 KETOSTEROIDS ANDROGENS AND ESTROGENS BY NORMAL CHILDREN AND ADULTS

Age in years	Boys			Girls		
	17 Ketosteroids mg	Androgens IU	Estrogens IU	17 Ketosteroids mg	Androgen IU	Estrogens IU
3–4			ca 10			ca 10
4–5	0 15–0 50			0 15–0 5		
5–6	(1 0)			(1 0)		
6–7						
7–8	0 65		20–25	0 65		20–25
8–9	0 95			0 95		
9–10	1 4	1–2		1 4	0 7–3 0	
10–11	1 9			1 9		
11–12	2 6			2 6		90
12–13	3 4	—		3 4		250
13–14	4 3	1–15	*25–35–60*	4 3	7	190
14–15	5 3	7–16		5 3		380
15–16	6 3	6–32	18–65	6 3		
16–17	7 2	14–19	30–95	7 2		
17–18	8 1	—	—	8 1		
Adult	11 0	10–110 (55–68)	10–100	6–8	5–10	50–300

Androgens 1 IU (international unit) = 0 1 mg of androsterone
Estrogens 1 IU = 0 1 μg of estrone; 1 MU (mouse unit) = 3 5 IU; 1 RU (rat unit) = 3 IU
After Seckel[34] and Sohval[35]

tropin from the 10 19 year decade through the 40 49 year decade in males In the next decade 50 59 years another slight increase is observed thus bringing the urinary gonadotropin value to approximately 12 5 units/24 hours After this period of life the *urinary gonadotropins exhibit a wide array of fluctuation* with values ranging from 9 to 25 In general it could be said that in men a slight increase in gonadotropin occurs as a function of age This slight but significant increase is less than half that of women at the comparable age span 10 through 49 years and approximately 20 per cent that of women over the comparable age span of 50 through 89 years[1]

b Androgens

The biological assay of androgens is fraught with numerous difficulties Therefore it is extremely difficult to state the exact androgen values for any specific age group The variation in the mean values of androgens may be anywhere from threefold to tenfold (19 99 I U per day and individual values from 10 to 255 I U) Dorfman and Shipley state that the wide variation within a laboratory or from laboratory to laboratory may be due to the differences in and/or completeness of extraction It is well known that during hydrolysis if the principal urinary androgen androsterone is converted to Δ or Δ^3 androstene 17 one as much as a tenfold loss *of androgenic activity usually results*[11]

From rather careful approximations it can be said that the androgenic activity in males rises from 1 2 *I U from 7 to 12 years of age to 1 15 I U during* the thirteenth to fourteenth year of life From 14 to 17 years a marked increase over that of females is observed 7 32 I U in males as opposed to *7 I U* in females The most dramatic increase in urinary androgens in males (6 32 I U /24 hours) is found during ages 15 16 This may be truly related to the crescendo of pituitary testicular activity associated with the establishment of sexual maturity or the regulation of the reciprocal pituitary testicular hormonal relationships After this sudden spurt of androgenic activity the urinary androgens reach an all time high of approximately 55 68 I U (Table 3) This value (55 68 I U) is interpreted as the so called normal average value of the adult male[34, 3]

c 17 Ketosteroids

These substances increase as a function of age During childhood the urinary 17 ketosteroids rise from an average of 1 0 to 3 4 mg daily A progressive increase up to 8 1 mg is observed during the period

The masculine attributes (body build deep voice facial hair and the male type escutcheon) are all affected by the circulating androgens

Extragonadal effects of the androgens have been reported Numerous investigators claim that androgens stimulate the enlargement of breasts but it must be emphasized that such occurs only under hypogonadal conditions in the male It has not been established what role endogenous androgens play in the transitory mammary enlargement observed in some boys during their period of adolescence Sohval believes that the administration of androgens to normal men does not result in breast enlargement indicating that the effect of androgenic hormone on the human male depends largely if not entirely upon the state of gonadal function

Protein anabolism and nitrogen retention as indicated in the introductory remarks are subject to the action of the circulating androgens The administration of testosterone to normal men induces about 50 per cent as much nitrogen retention as it does in eunochoids the greatest effects being observed in younger rather than older men Testosterone also exerts a myotropic effect in hypogonadal men It has been known since the pioneer researches of F Albright and E C Reifenstein Jr that testosterone is quite useful in the treatment of osteoporosis Testosterone displays a high index of protein anabolic activity and is quite effective in increasing the protein matrix of bone [9]

Creatine metabolism is also influenced by testosterone It is well known that methyltestosterone produces creatinuria in dwarfed boys and girls Recent evidence indicates that the C 17 methylated androgens stimulate both synthesis and storage of creatine Testosterone propionate seems to exert its effect only on the storage process [88]

Renal effects have been ascribed to androgenic activity but most of the available data are from laboratory animals There is however circumstantial evidence pointing to a possible relationship between androgens and kidney size The kidneys of the adult human male are approximately 10 per cent greater than those of the female Experimental evidence from male rats and mice indicates that testosterone influences kidney size and weight Kidneys in male mice and rats normally decrease in weight after orchidectomy The administration of androgens to orchidectomized rodents prevents the decrease in kidney size

Water balance and electrolytes are markedly influenced by androgens Testosterone induces a retention of sodium chloride potassium inorganic phosphorus and water It has been reported that in the treatment of mammary carcinoma testosterone displays a hypercalcemic effect Retention of water and salt usually occurs in eunuchoids when treated with testosterone propionate over long periods of time Indiscriminate or chronic use of androgens in older patients with cardiovascular disease may result in edema and cardiac failure

Erythrocytes percentage of hemoglobin and hematocrit level are all increased as a result of androgen therapy in orchidectomized rodents and hypogonadal men These effects are usually quite remarkable in eunuchoids without pituitary disease Such effects have not been observed in normal men

Voice skin mucous membranes and hair are all affected but to different degrees by the circulating androgens *and* genetic tendencies The administration of androgens to hypogonadal males will correct in large measure their sallow complexions inability to sun tan and lack of facial hair Normally it is believed that androgens display a characteristic effect on the mucosa of the laryngeal membranes and vocal cords thus regulating the depth of the voice The hair of the face scalp (frontotemporal hairline) and pubic area are likewise stimulated to grow by the combined androgenic (of *adrenal* and *testicular* origin) and genetic effects

G NEW SYNTHETIC SEX HORMONES

The recent literature is replete with basic and clinical information concerning a number of synthetically produced sex hormones which display estrogenic or progestational activity

1 Estrogens

Numerous synthetic steroidal and non steroidal estrogens have been described and evaluated clinically These include diethylstilbestrol dienestrol hexestrol benzestrol triphenylethylene tri paraanislychorethylene and Delestrogen All of these have been found to be quite active in the human female Diethylstilbestrol possesses about the same magnitude of estrogenic activity as the naturally occurring estrogen from the theca interna estradiol 17β Delestrogen estradiol 17 n valerate an esterified estrogen is quite potent and also displays prolonged estrogenic activity in the human female

2 Progestins (Gestagens)

Clinical evidence indicates that there are several synthetically produced and esterified progestins which display a greater progestational activity than progesterone Delalutin (17 alpha hydroxy progesterone 17 n caproate) has been reported to be a potent long acting progestational compound with an important therapeutic role in obstetrical complications especially in recurrent abortion Reifenstein and thirty of his clinical collaborators have reported rather convincing data indicating that Delalutin is more advantageous than other progestational substances because

that progesterone acts upon the reproductive tract *after* estrogen has had the first opportunity to do so As a result and if one examines the actions of the estrogens it is generally believed that estrogens are concerned with the growth and partial development of the female reproductive tract whereas the combination (and in some instances the synergistic actions) of the estrogenic and progestational hormones are responsible for the full development and differentiation of the female reproductive organs In any event it seems quite clear that purified progesterone *alone* does not induce the so-called progestational changes but rather does so only after estrogen induces its growth effect first

Metabolic changes are also induced by progesterone *In general it is believed that progesterone* has the ability to retain sodium and water and induce weight increases Combinations of estrogens and progestins induce a catabolic effect in normal males and females by increasing urinary excretion of nitrogen sodium and chloride In lower mammals (cats dogs rats and mice) it has been shown that progesterone alone can substitute for the life-maintaining properties of the adrenal hormones Much less is known about progesterone in contrast to the estrogens in the human

The ovaries are influenced by progesterone but most of the available information is of a theoretical nature and is derived from experimental procedures in laboratory animals It is assumed that progesterone like estrogen may have two effects upon the ovaries stimulatory and inhibitory During the normal sequence of events it has been assumed that combinations of estrogen and progesterone induce a greater release of LH and a concomitant secretion of LtH from the adenohypophysis It is also believed that sufficient titers of progesterone restrict in a large measure the release of LH and simultaneously effect an outpouring of FSH required for the continuing cyclic ovulatory process

The uterine tubes display less muscular tonus during the progestational phase and it is believed that progesterone reduces in part the contractions observed during the follicular phase

The endometrium as a result of the action of progesterone undergoes a transformation from the so called proliferative phase to the secretory phase The endometrium of the progravid phase contains enlarged glands and these appear highly tortuous in character The surface and the glandular epithelium display intense amounts of glycogen and mucopolysaccharides at this time Microscopic examination of the progravid endometrium reveals that there is a large amount of secretory material in the glandular lumina The spiral arterioles display a greater coiling and appear somewhat enlarged when compared with those of the proliferative phase The combined actions of the estrogens and progestational substances produce a synergistic growth effect on the endometrium the endometrial stromal cells are greatly enlarged the width of the endometrium is thickened and after ovulation (16-22nd day of a typical 28-day menstrual cycle) the endometrium is truly prepared to become the environment for the fertilized ovum Should fertilization and nidation not occur the corpus luteum regresses and there is a concomitant reduction in the release of progestins and estrogens Menstruation the sloughing-off of the functionalis of the endometrium normally results *following the regression of the corpus luteum and* reduction of ovarian hormones during the normal cycle A more detailed discussion of the clinical concepts of menstruation appears in Chapter III

Uterine contractions and myometrial growth are both affected by progesterone The spontaneous contractions normally observed during the proliferative phase are decreased as a result of the action of progesterone

The cervix likewise responds to progesterone Cervical secretions are much less in amount during the progestational phase and the glycogen content of the tissue is also reduced

The vaginal epithelium is thickened and undergoes a marked change as a consequence of progestational action Progesterone effects a diminution of cornified cells and induces a clumping of these cells The non cornified cells appear folded and display slightly turned in edges thus giving an appearance of being curled

The mammary glands require progesterone in addition to a number of pituitary hormones for their complete development and functional activity While estrogen is required for ductal growth *and* stromal proliferation progesterone and other hormones act upon the initial growth stimulus of the estrogens and induce lobular alveolar proliferation Reece has recently reviewed and critically evaluated our information on mammary gland development and function

2 Androgenic Actions in the Male

Androgens have as their principal over all metabolic effect the property of accelerating protein synthesis

The male reproductive system and its associated structures are dependent upon the male sex hormones for their development and function These include the internal genitalia (prostate seminal vesicles and Cowper s glands) and the external accessory genitalia (penis scrotum epididymis vas deferens and glands of Littre)

The recent work by Dr Bartelmez suggests that the designation *progravid* is more correct than *secretory* for that phase of the cycle following ovulation

37 Smith R A and Albert A 1956 Effects of progesterone on urinary gonadotropin Proc Staff Meetings of Mayo Clinic May 16 1956 31 309 11
38 Sohval A R 1958 Anatomy and endocrine physiology of the male reproductive system The Endocrinology of Reproduction ed J T Velardo Oxford Univ Press New York 243-312
39 Sternberg W H Segaloff A and Gaskill C J 1953 Influence on human ovarian hilus cells (Leydig like cells) J Clin Endocrinol & Metab 13 139-53
40 Velardo J T 1957 Action of adrenocorticotropin on pregnancy and litter size Amer J Physiol 191 319 22
41 —— 1957 Influence of purified adrenal hormones and adrenocorticotropin on progesterone in decidual tissue formation Amer J Physiol 190 408 12
42 —— 1958 Anatomy and endocrine physiology of the female reproductive system The Endocrinology of Reproduction ed J T Velardo Oxford Univ Press New York 101 212
43 Velardo J T editor 1958 The Endocrinology of Reproduction Oxford Univ Press New York 339 pp
44 Velardo J T Raney N M Smith B G and Sturgis S H 1956 Effects of various steroids on gestation and litter size in rats Fertility & Sterility 7 291-311
45 Velardo J T and Sturgis S H 1955 Interaction of 16 Epi Estriol and Estradiol 17β on uterine growth Proc Soc Exp Biol & Med 90 609 10
46 —— 1956 Suppression of uterine growth by purified hydrocortisone acetate 9 alpha fluoro-hydrocortisone acetate and corticotropin J Clin Endocrinol & Metab 16 496-506
47 Zander J Forbes T R Neher R and Desaulles P 1957 Über biologisch aktive progesteron metaboliten im Menschlichen organismus Klin Woch 35 (3) 143
48 Zarrow M X Holmstrom E G and Salhanick H A 1955 The concentration of relaxin in the blood serum and other tissues of women during pregnancy J Clin Endocrinol & Metab 15 22 7

of its longer duration of action and greater solubility thus making it possible to inject large amounts of this progestational substance [31] Norlutin (17 alpha ethynyl 19 nortestosterone) Enovid (composed principally of 17 alpha ethynyl 17 hydroxy 5(10) estren 3 one it is a brand of norethynodrel with ethynyl estradiol 3 methyl ether) and Prodox (17 acetoxy progesterone) have been reported to be quite effective when administered orally as progestational agents The recent availability of these progestational compounds thus makes it possible to deal more effectively with pregnancy complications in the human female

BIBLIOGRAPHY

1 *Albert A Randall R V Smith R A and Johnson C E* 1956 Urinary excretion of gonadotropin as a function of age In Hormones and the Aging Process ed Engle E T and Pincus G Academic Press Inc pp 49 62 New York
2 Austin C R 1948 Number of sperms required for fertilization Nature 162 534
3 ——— 1949 Fertilization and the transport of gametes in the pseudopregnant rabbit J Endocrinol (British Empire) 6 63 70
4 Austin C R and Braden A W H 1952 Passage of the sperm and the penetration of eggs in mammals Nature 170 919
5 Austin C R and Smiles J 1948 Phase contrast microscopy in the study of fertilization and early development of the rat egg J Roy Micr Soc (British Empire) 68 13
6 Baggett B Engel L L Savard K and Dorfman R I 1956 The conversion of Testosterone 3 C^{14} to C^{14} estradiol by human ovarian slices J Biol Chem 221 931-41
7 Birnberg C H and Abitbol M M 1957 Refined relaxin and length of labor Obst & Gynec 10 366 70
8 Blandau R J and Odor D L 1952 Observations on sperm penetration into the ooplasm and changes in the cytoplasmic components of the fertilizing spermatozoon in rat ova Fertility & Sterility 3 13
9 *Coffin H C and van Dyke H B 1941 Proposed* names for the follicle stimulating and interstitial cell stimulating hormones of the anterior lobe of the pituitary body Science 93 61
10 Dorfman R I and Goldsmith E D (Conference Co-Chairman) 1951 The influence of hormones on enzymes Annals N Y Acad Sci 54 531 728
11 *Dorfman R I and Shipley R A 1956 Androgens* Biochemistry Physiology and Clinical Significance New York John Wiley & Sons Inc 590 pp
12 Forbes T R 1950 Systemic plasma progesterone levels during the human menstrual cycle Amer J Obst & Gynec 60 180 86
13 Fraser R W Forbes A P Albright F Sulkowitch H and Reifenstein E C Jr 1941 Colorimetric assay of 17 ketosteroids in urine J Clin Endocrinol 1 234 56
14 Gallagher T F 1956 Secretion and metabolism of androgens Endocrines and Cancer Annual Scientific Session Oct 29 30 New York The American Cancer Society Inc p 9 (abs)
15 Gallagher T F Peterson D H Dorfman R I Kenyon A T and Koch F C 1937 The daily urinary excretion of estrogenic and androgenic substances by normal men and women J Clin Invest 16 695 717
16 Greenwald Gilbert S 1956 Sperm transport in the reproductive tract of the female rabbit Science 124 586
17 Greep R O and Jones I C 1950 Steroid control of pituitary function In Recent Progr in Hormone Research ed Pincus G New York Academic Press Inc Vol V 197 261
18 Greer M A 1957 Studies on the influence of the central nervous system on anterior pituitary function In Recent Progr in Hormone Research ed Pincus G 67 104 New York Academic Press Inc
19 Hartman C G 1957 How do sperms get into the uterus? Fertility & Sterility 8 403 27
20 Hisaw F L Velardo J T and Goolsby C M 1954 Interactions of estrogens on uterine growth J Clin Endocrinol & Metab 14 1134-43
21 Hisaw F L Velardo J T and Ziel H K 1951 Action of pregnanediol and related steroids on progesterone in decidual development J Clin Endocrinol & Metab 15 763
22 Hisaw F L and Zarrow M X 1951 The Physiology of relaxin Vitamins and Hormones ed Harris R S and Thimann K V Academic Press 8 151 New York
23 Hotchkiss R S 1944 Fertility in Men Philadelphia J B Lippincott
24 Leiberman S and Teich S 1953 Recent trends in the biochemistry of steroids Pharm Rev 5 285 3?0
25 Mann T 1954 The biochemistry of semen London Methuen & Co New York John Wiley & Sons Inc 240 pp
26 Markee J E and Berg B 1944 Cyclic fluctuations in blood estrogen as a possible cause of menstruation Stanford Med Bull 2 55 60
27 Marrian G F 1957 The urinary estrogens and their quantitative determination Cancer 10 704 6
28 Parkes A S editor Marshall s Physiology of Reproduction Third edition Vol I Part I 1956 Vol II 1952 London Longmans Green and Co
29 Rakoff A 1958 Summary of conference on new steroid compounds with progestational activity Ann N Y Acad Sci In Press
30 Reifenstein E C Jr 1957 Introduction of marked as well as prolonged biologic activity by esterification 17 alpha hydroxyprogesterone caproate a unique progestational compound Fertility & Sterility 8 50
31 ——— 1958 The clinical use of 17 alpha hydroxy progesterone 17 n-caproate in habitual abortion Ann N Y Acad Sci In Press
32 Rezek C H 1953 The effect of a new potent uterine relaxing factor of the corpus luteum in the treatment of dysmenorrhea Amer J Obst & Gynec 66 396 402
33 Salhanick H A Hisaw F L and Zarrow M X 1952 The action of estrogen and progesterone on the gonadotropin content of the pituitary of the monkey J Clin Endocrinol & Metab 12 310 20
34 Seckel H P C 1946 Precocious sexual development in children Med Clinics of No Amer 30 183 209
35 Smith O W and Smith G V 1952 Endocrinology and related phenomena of the human menstrual cycle Recent Progr in Hormone Research Vol II ed Pincus G New York Academic Press Inc Ch 7 pp 209 53
36 *Smith R A and Albert A 1955 Effect of estrogen on urinary gonadotropin* Proc Staff Meetings of Mayo Clinic December 28 1955 30 617 20

a passive type of migration In this connection it related to the growth of the surrounding tissues Everett[81] in 1943 likewise pointing out the short distance between the gut entoderm (where in the mouse embryo he also identified germ cells) and the genital ridge stated I see no reason why a germ cell cannot be carried this short distance by a shifting of the tissue or by its own power or by both means By transplantation experiments Everett furnished crucial proof of the extragonadal source of germ cells in the mouse

In 1948 Witschi[30] in a comprehensive study of human embryos ranging from the 13 somite (3 5 mm) to the 8 mm stage corroborated the initial findings of Fuss In summarizing his results Witschi remarked

In the youngest embryo all germ cells are in the endoderm of the yolk sac near the allantoic evagination this observation indicates that the germ cells either have an endodermal origin or arise from primitive stem cells which also are the source of the endoderm

In embryos of from 13 to 20 somites [which do not exceed 3 5 mm in length i e during the first three weeks of development] the germ cells become transferred from the yolk sac to the hind gut A variable number -probably always less than half— remain temporarily in the endodermal epithelium The larger number leave the endoderm and move into the mesenchyme

The second phase of migration begins in embryos of more than 25 somites The germ cells leave the gut ascend through the mesentery and advance laterally toward the mesonephric folds [At this stage the embryo has attained a length of about 5 mm (c 28 days ovulation age)]

During this phase the germ cells form lobate and filiform pseudopodia and give signs of lytic action It is concluded that migration is accomplished by active movements of the individual cells

More recently McKay Hertig Adams and Danziger[154] utilizing a special histochemical technique have furnished additional evidence supporting the validity of the observations of Fuss and Witschi in regard to the extragonadal origin of the germ cells in man However with respect to the mode of transfer of the goma to the gonadal site McKay[153] favors the second mechanism proposed by Fuss[95] (passive) rather than the first one (active by ameboid movements) which was also advocated by Witschi in 1948 As McKay has expressed it "This so called migration of the extragonadal germ cells may actually represent the process of differential growth rate of surrounding tissues with consequent displacement (pushing and pulling) of the germ cells

B Gonadogenesis * The Indifferent Stage of the Gonad

Whatever the method of positioning of these moderate sized primordial germ cells McKay and his associates[154] have demonstrated by a special stain that in a 5 mm human embryo (c 28 days ovulation age) the developing gonadal folds serve as their resting place At this stage the male and female genital folds are histologically identical (Cf Figs 1 and 2[154])

While some of these primordial germ cells are believed to remain in the thickened celomic membrane to help organize the germinal epithelium at

* For the following attempt to formulate our concept of gonadogenesis and ovariogenesis we have drawn largely from studies of Dr Donald C McKay and his co workers and we are furthermore indebted to Dr McKay for his kindly personal assistance



Fig 1 Section through the ovary of a 24 cm fetus (BLI S57 10) of c 25 weeks ovulation age Primordial germ cells and nurse cells from the mesenchyme which accompany the germ cells into the genital ridge These two types of cells are almost indistinguishable from each other in the outer cortical area (right upper half of photograph) However as the germ cells approach the hilus (left and lower right) they enlarge to become primordial ova and are now clearly differentiated from the nurse cells which arranged around the peripheries of the respective primordial ova appear as much smaller epithelioid cells (×400)

II

Ovulation

CELSO RAMON GARCIA M D
AND
JOHN ROCK M D

Mulier est quod ovarium est said Virchow[12 112] Surely a woman is what her ovaries make her for they must perform her biological role discharge of ova insurance of their fertilization by her femininity nourishment of their product and finally safe de liverance of young Ovulation is the beginning

Before discussing this important function it may be well to consider briefly the process of oogenesis i e the origin of ova and the transitional stages by which some of them reach maturation and release

I THE PREPARATION FOR OVULATION OOGENESIS

The embryology of the human gonad was recently described by F D Allan[4] The intrinsic complexity of some aspects leads us to review them here

A Origin of the Primordial Germ Cells

In spite of the fact that the germ cells are indis pensable to the species there is still no agreement among the various investigators in the field as to whence they come In his scholarly survey on the source of the germ cells Everett[82] has discussed the various theories on the basis of four major divisions the categorization of which he credits to Florence Heys (Cf her summary page 41 *Quart Rev Biol* Vol 6 1931) However as this author[81] had ex pressed it in a previous publication The fundamen tal question to be answered and one which has been responsible for these differences in opinion is whether the definitive germ cells are derived from undifferentiated cells which are set aside early in development and which later migrate to the gonad or whether they are merely transformed soma cells which originate in the gonad from time to time even in the adult

Thus throughout the years the chief controversy has centered about the intragonadal *vs* extragonadal origin of these essential units and even today the problem still remains unsettled

1 INTRAGONADAL ORIGIN OF THE GERM CELLS (WALDEYER'S THEORY)

In 1870 Waldeyer[2 6] proposed that all such cells arise from the germinal epithelium which he iden tified as a thickening of the celomic epithelium to form the mesonephric bodies in the region extend ing from segments T 8 to L-4 According to his view these primordial germ cells later migrate into the ovary and constitute the progenitors of all the definitive ova that a particular woman will possess throughout her entire life

Whether this proliferation from the germinal epi thelium is limited to the fetal period or continues throughout a woman's child bearing years constitutes the subject of a second controversy in this field which will be discussed later in this chapter

2 EXTRAGONADAL ORIGIN OF THE GERM CELLS

The alternate viewpoint is the so called *Keimbahn* (Germ Track) Theory This is based on the concept advanced by Weismann [9] which he derived from his experimental studies on the hydromedusae Weis mann maintained that early in the development of an individual there is a distinct separation of the germ cells from the so called *soma* or body cells thus resulting in a direct continuity of germ cells from each generation to the next Direct evidence that such is the case in the human was first presented by Fuss[9 4] who in 1912 demonstrated that human embryonic cells later to be recognized as oogonia (or spermatogonia) arise in the entoderm of the yolk sac lateral to the posterior portion of the embryonic blastoderm Fuss[95] proposed that the germ cells migrate through the yolk sac entoderm and mesen chyme to the gonad forming area in part by virtue of active ameboid movements Fuss also postulated

a passive type of migration in this connection i e related to the growth of the surrounding tissues Everett[81] in 1943 likewise pointing out the short distance between the gut entoderm (where in the mouse embryo he also identified germ cells) and the genital ridge stated I see no reason why a germ cell cannot be carried this short distance by a shifting of the tissue or by its own power or by both means By transplantation experiments Everett furnished crucial proof of the extragonadal source of germ cells in the mouse

In 1948 Witschi[30] in a comprehensive study of human embryos ranging from the 13 somite (3 5 mm) to the 8 mm stage corroborated the initial findings of Fuss In summarizing his results Witschi remarked

In the youngest embryo all germ cells are in the endoderm of the yolk sac near the allantoic evagination this observation indicates that the germ cells either have an endodermal origin or arise from primitive stem cells which also are the source of the endoderm

In embryos of from 13 to 20 somites [which do not exceed 3 5 mm in length i e during the first three weeks of development] the germ cells become transferred from the yolk sac to the hind gut A variable number—probably always less than half—remain temporarily in the endodermal epithelium The larger number leave the endoderm and move into the mesenchyme

The second phase of migration begins in embryos of more than 25 somites The germ cells leave the gut ascend through the mesentery and advance laterally toward the mesonephric folds [At this stage the embryo has attained a length of about 5 mm (c 28 days ovulation age)]

During this phase the germ cells form lobate and filiform pseudopodia and give signs of lytic action It is concluded that migration is accomplished by active movements of the individual cells

More recently McKay Hertig Adams and Danziger[54] utilizing a special histochemical technique have furnished additional evidence supporting the validity of the observations of Fuss and Witschi in regard to the extragonadal origin of the germ cells in man However with respect to the mode of transfer of the gonia to the gonadal site McKay[153] favors the second mechanism proposed by Fuss[95] (passive) rather than the first one (active by ameboid movements) which was also advocated by Witschi in 1948 As McKay has expressed it This so called migration of the extragonadal germ cells may actually represent the process of differential growth rate of surrounding tissues with consequent displacement (pushing and pulling) of the germ cells

B Gonadogenesis The Indifferent Stage of the Gonad

Whatever the method of positioning of these moderate sized primordial germ cells McKay and his associates[154] have demonstrated by a special stain that in a 5 mm human embryo (c 28 days ovulation age) the developing gonadal folds serve as their resting place At this stage the male and female genital folds are histologically identical (Cf Figs 1 and 2[154])

While some of these primordial germ cells are believed to remain in the thickened celomic membrane to help organize the germinal epithelium at

* For the following attempt to formulate our concept of gonadogenesis and ovanogenesis we have drawn largely from studies of Dr Donald G McKay and his co-workers and we are furthermore indebted to Dr McKay for his kindly personal assistance



Fig 1 Section through the ovary of a 24 cm fetus (BLI S57 10) of c 25 weeks ovulation age Primordial germ cells and nurse cells from the mesenchyme which accompany the germ cells into the genital ridge These two types of cells are almost indistinguishable from each other in the outer cortical area (right upper half of photograph) However as the germ cells approach the hilus (left and lower right) they enlarge to become primordial ova and are now clearly differentiated from the nurse cells which arranged around the peripheries of the respective primordial ova appear as much smaller epithelioid cells (× 400)

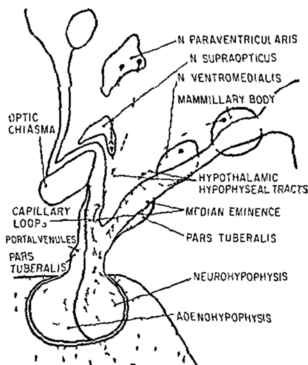


FIG 2 DIAGRAM REPRESENTING MEDIAN SAGITTAL SECTION OF HUMAN HYPOTHALAMUS AND HYPOPHYSIS The broken lines indicate only the hypothalamic hypophyseal portal elements of the hypophyseal blood supply Reproduced by permission from Cleghorn R A and Graham B F 1949 Manifestations of altered autonomic and humoral functions in psychoneuroses In *Recent Progress in Hormone Research* *The Proceedings of the Laurentian Hormone Conference* Edited by G Pincus 4 323 62 Academic Press Inc New York N Y

about the 22 mm stage (*c* 42 days ovulation age) most of them have reached beyond the germinal epithelium in association with accompanying mesenchymal cells The mesenchymal cells extend into the cortex as the so called cords of Pfluger within which the germ cells having diminished in size are for a time almost indistinguishable from the nurse cells that differentiate from the mesenchyme After the first few (*c* 6) embryonic weeks the germ cells again enlarge to become recognized as *primordial ova*

C Ovariogenesis The Primordial Ovum

According to McKay[154] at about the 22 mm stage (*c* 42 days *ovulation age*) it is possible to distinguish between testis and ovary The ovary at this time consists essentially of a solid mass of primordial germ cells and mesenchymal interstitial cells covered by a cuboidal layer of celomic epithelium Differentiation without division of the primordial germ cells into primordial ova begins in the cells nearest the hilus of the ovary During this process other primordial germ cells (oogonia) are continuing to divide

Various other interpretations as to the composition and role of the cords of Pfluger have been advanced These are ably summarized by Sauramo[18]

Sauramo[18] who has made a detailed study of oogenesis at all stages of prenatal life states that in the fifth month of gestation when the fetus has attained a size of 19 cm these primordial ova begin to be enclosed within the so called nurse cells thus forming *primordial follicles* Division of oogonia has ceased by the sixth month[244]

Figure 1 shows a section of an ovary at a slightly later stage (24 cm in length ovulation age of approximately 25 weeks) The outer portion of the ovarian cortex is occupied by clusters of primordial germ cells and the inner portion by primordial ova each ovum being surrounded by a single layer of nurse cells These arise from the mesenchymal interstitial cells (Pflugers cords) contiguous to the germ cells which they accompanied into the gonad The nurse cells constitute the anlage of the future *granulosa* During this stage some primordial ova are still without surrounding nurse cells

Before it is enclosed by a layer of nurse cells the primordial ovum is said by Sauramo[218] to average about 20 μ in diameter (16 25 μ) while the primordial follicle reaches a size of approximately 30 μ[44] For a detailed account of the sizes of ova and follicles at different ages during the child bearing period the reader is referred to Sauramos[217] excellent presentation

During the last two months of intra uterine life the wave of differentiation spreads outward and in the term infant the germ cells have virtually all been converted into primordial ova According to Sauramo[7] not all of these are within primordial follicles at birth for he still found some unenclosed primordial ova in the two-month old infant By the age of six months however primordial ova are no longer present as such all being within follicles

D Life History of the Primordial Follicles

1 NUMBER PRESENT AT DIFFERENT AGES

The number of primordial follicles in both ovaries at birth has been estimated by various authors to be as low as 40 000[91] and together with free oocytes as high as 600 000[2] In his study of the human ovary from birth to sexual maturity Simkins[4] calculated the number of primordial follicles in one ovary at different ages these calculations ranged from 143 000 in the newborn infant to 10 500 at 14 years Haggstrom[10] found the total number of follicles in both serially sectioned ovaries of a 22 year old woman to be about 432 000 Of these approximately

12 000 were atretic Of the approximately 420 000 nonatretic follicles nearly 390 000 were less than 50 μ in diameter while about 1 700 others showed signs of beginning development Only 219 additional ones had reached the stage of antrum formation

The report of Block published in 1952 (Acta Anat 14 108 23) like that of Simkins [44] mentioned above indicates a decrease in the number of primordial follicles with advancing age This author counted the primordial follicles in both ovaries of 43 females at different age levels Dividing his specimens into age groups at 10 year intervals Block noted a striking decline with age in the number of primordial follicles ranging from 439 000 in the 6-15 year group to 34 900 in the ovaries of women age 36 through 45 years However in spite of this apparently clear-cut effect of age as shown by the average figures Block draws attention to the tremendously wide variation in the number of primordial follicles among *individual* members of a given age group

In seven of the oldest women of the series (ages 39 through 45) Block found the number of primordial follicles in both ovaries to range from 350 to 28 000 with a mean of 8 300 thus providing additional evidence that as the menopause is approached and the cortex becomes thinner there may be very few primordial follicles in the ovary

2 Theories of Ovular Quantitation

The decrease in the number of primordial follicles from birth to the climacteric has lent credence to the so called stock pile theory of oogenesis This proposes that all ova later present in the adult ovary were there as the primordial germ cells which developed without division into primordial ova when the gonad was first recognizable is such under the modified celomic (germinal) epithelium of the genital ridge As already mentioned this occurs at some indeterminate time just before the 22 mm stage (c 42 days ovulation age)

The other theory that of continuous oogenesis maintains that from birth to the climacteric the germinal epithelium is constantly in cyclic fashion producing new cells that give rise to ova and follicle cells

The frequency of atretic follicles in normal adult ovaries shows clearly that at least many if not all the ova which closely approach but fail to reach maturity during a specific cycle degenerate According to the continuous production theory the losses as a result of this atresia are replaced in succeeding cycles by the growth of new primordial ova and nurse cells from those embryonic germ cells which may have remained in the germinal epithelium at the end of their initial shift of position from the entoderm of the yolk sac

Although the germinal epithelium in the testis of the male is known to produce spermatogonia continuously throughout adult life [9] the homologous stages in the process of oogenesis have not been clearly elucidated

Studies of Allen [6] on the mature mouse and of Evans and Swezy [79] on the cat dog guinea pig and rat led these scholars to believe that in the above mentioned species the germinal epithelium is constantly from birth to senescence giving rise to ova and nurse (follicle) cells These workers concluded that oogenesis varied in intensity with the phases of the estrous cycle Confirmation of their results was reported several years later by Allen and Creadick [7] and by Bullough [30 33] all of whom repeated these studies on the mouse subjected to colchicine which arrests mitosis in the metaphase and hence yields tissues containing more mitotic figures than in nontreated animals

The results and interpretations of the above mentioned investigators have been severely criticized by Mandl and Zuckerman [118] on two main grounds As these authors express it (1) the relationship between the mitotic activity of the germinal epithelium and oogenesis has never been unequivocally demonstrated and (2) the work has not been quantitatively controlled Furthermore after repeating these studies on the rat and utilizing statistic methods Mandl and Zuckerman failed to find any correlation between number of oocytes in the ovary and the stages of the estrous cycle In concluding they state Statistical analysis showed that age and litter relationship influence the total number of oocytes to an extent which completely overshadows any influence on the total count which might be exercised by the oestrous rhythm Their observations also suggested *that the rate of atresia likewise does not vary systematically* with the phases of the rat s vaginal cycle However they caution against applying their results on atresia noted in the rat to a species in which only a single ovum is released at ovulation time

In a comprehensive review of the entire subject Zuckerman [307] in 1951 presented nearly a dozen other lines of evidence mainly from animal experimentation which led him to conclude that the view that oogenesis continues throughout reproductive life is very insecurely based

In the human on the other hand Schwarz Young and Crouse [2 3] had stated on the basis of their study of oogenesis that the development of the new ova after birth and in active adult life can be definitely accepted and that the main source of these ova is the germinal epithelium Simkins [44] while also a proponent of the concept of continuous oogenesis had previously expressed the opinion that during

the child bearing years new ova are formed from cells in the ovarian parenchyma which are not to be considered as stored germ cells but somatic cells induced to grow into germ cells by the gonad stimulating hormone of the anterior lobe of the hypophysis

Whereas Zuckerman [807] cites the demonstration by Gaillard that human fetal ovarian germinal epithelial cells can produce ova in tissue culture as evidence of the oogenetic potentiality of germinal epithelium during prenatal life he points out that Parallel attempts to cultivate fragments of adult human ovaries provided no convincing evidence that there was any proliferation of epithelial elements Indeed even Schwarz [3] himself in the discussion following the presentation of his paper admitted that he had failed to observe *mitosis in the human ovary after birth* He had however seen evidence of division of ova by amitosis as had also (he said) Swezy and Evans and Simkins Hertig [118] on the other hand states that he has never seen evidence of oogenesis in the germinal epithelium of the adult human ovary

We therefore agree with Hertig's [11] concept that the aging of the ovary really begins at birth that there are no abrupt changes and that In general the aging takes place in an orderly and progressive fashion resulting in a gradual loss of primordial ova and their follicular phases a progressive thinning of ovarian cortex which becomes increasingly wrinkled or gyrated and a relative increase in the medulla which becomes the repository for corpora albicantia

After the age of 50 there is a rather striking loss of all phases of follicular activity One only rarely encounters evidence of follicular activity primordial or otherwise

For a more complete survey of this subject the reader is referred to the works of Pincus [183] Everett [81, 2] Moore and Wang [159] Zuckerman [807] Sauramo [18] and Shettles [35]

E Development of the Primordial Ovum (Follicle) into the Mature Ovum (Preovulatory Graafian Follicle)

In a scholarly review entitled Development of the Graafian Follicle and Ovulation Hisaw [1 0] in 1947 analyzed step by step on an endocrine basis the events in the transformation of the primordial ovum which culminate in the bursting of the ripe follicle and the release of its egg While some of Hisaw's premises even today lack adequate experimental proof his account still remains the most satisfactory explanation for this process Taking into consideration more recent data acquired in the field during the past ten years as well as our own interpretations we now visualize as follows four stages in follicular development approximately as conceived by Hisaw

1 Control by Ovum Itself and Granulosa Later by Estrogen

(a) Stage I

One may consider as a starting point that as early as the fifth month of gestation (Sauramo [18]) the primordial follicle consisting of an oogonium a primordial ovum surrounded by a single flat layer of nurse cells derived from the mesenchymal elements in Pfluger's cords has a self contained system of organizers which controls its growth As the oogonium increases in size even during late intra uterine tenancy by the embryo the surrounding follicular cells become cuboidal This is the first definitive stage in the developmental process following the evolution of the primordial follicle described above as occurring in the hilus of the fetal ovary At successive times and well into the menopause determined by unknown forces which Hisaw suggests are self contained these epithelioid (nurse) cells in discrete follicles here and there in the ovary proceed to *increase in number by mitosis forming the multilayered granulosa* From the adjacent stroma the connective tissue cells likewise multiply and differentiate into the *theca interna* which becomes part of the follicular entity All this Hisaw proposes is accomplished under the influence of the ovum and its surrounding granulosa cells

In the year following Hisaw's publication of this concept of an intra ovular self contained system of organizers which he postulated as the regulator of follicular growth Vincent and Dornfeld [4] presented evidence strongly suggesting that this regulator might well be ribonucleic acid Their results led these workers to remark it may be conjectured that the early growth of the granulosa is stimulated by ribonucleic acid diffusing from the rapidly enlarging oocyte It may likewise be suggested that the organization and initial growth of the theca traces to the ribonucleic acid diffusing outward from the granulosa

The theca interna a multilayered tissue composed of cells which are granular in appearance due to the presence of fat and yellowish pigment is moderately *vascular and contains* numerous lymphatics From the theca interna estrogen probably in the form of *estradiol* [247] is secreted by virtue of its own intrinsic enzyme system and this hormone now becomes the indispensable self organizer of further development The estrogen produced in the theca and demonstrated by both *biologic* [304 48 303] *and histochemical* [63 65 62] methods now governs the elaboration of estrogen containing fluid by the granulosal layer The expansion of this so called *liquor folliculi* results in a slightly distended area located eccentrically and lined

by several rows of cells the *membrana granulosa* With the appearance of this newly formed incipient *antrum* we have the beginning of the *graafian follicle* the establishment of which marks the end of Hisaw's first stage

2 Control by Gonadotropic Hormones of the Anterior Pituitary Gland

From now on the further development of the follicle is under the influence of the three gonadotropic hormones of the anterior pituitary gland *FSH LH* and *LtH*[267 301] As the gonadotropic hormones react on the ovary the latter in turn reacts on the anterior pituitary gland thus leading to an orderly regulation in the sequence of cyclic changes These reactions and counter reactions have been clearly elucidated by Fevold[85]

(a) stage II

After reacting to its own estrogen as described above the follicle gains competence to respond at the onset of Stage II to the *follicle stimulating hormone (FSH)* of the anterior pituitary gland The preliminary preparation of the follicle by estrogen in this fashion is fundamental and only those follicles sufficiently activated by it will maturate FSH is the substance responsible for initiating enlargement of the vesicle but this occurs only after estrogen has primed the granulosa and possibly the theca also

This estrogen from the enlarged follicle has endocrine targets other than its initial one i e its own follicular wall for directly or indirectly it gradually causes the pituitary gland to begin secreting *luteinizing hormone (LH)* synonymous with *interstitial cell stimulating hormone (ICSH)* Under the influence of two of the gonadotropic hormones (FSH and LH) the follicle increases in size and assumes an ovoid shape while augmenting its estrogen production

As the follicular cells multiply and the follicular cavity enlarges secondarily to the increase in its fluid the accumulation of the fluid keeps the ovum to one side Now eccentrically placed it becomes surrounded by an extension of granulosal cells projecting into the fluid cavity This enclosure containing the ovum is termed the *cumulus oophorus discus proligerus* or *germ hill* The remainder of the follicular cavity proper is well lined by the membrana granulosa which is now composed of several layers of cuboidal or polyhedral cells The inner cells of the cumulus oophorus become columnar and arrange themselves in regular radiating layers around the ovum to form the *corona radiata*

(b) stage III

Hisaw's third stage begins with the well developed follicle already producing appreciable amounts of estrogen As this third stage progresses even more estrogen is secreted and by virtue of more pituitary LH synergizing with FSH gradually increasing but minute amounts of progesterone are elaborated This is secreted by the theca interna [98] as the follicle extends its growth toward maturity With the accumulation of follicular fluid the follicle within its luteinized theca interna may attain a diameter of the order of 5 or even 8 mm at this stage

(c) stage IV

This is the short preovulation phase terminated by rupture In addition to FSH and LH the follicle at the end of Stage III acquires sensitivity to a third pituitary gonadotropic hormone *prolactin lactogenic hormone* or *luteotropic hormone (LtH)*[14 13 169 74] This hormone has slightly different chemical properties from LH and (on the basis of animal studies) seems to be associated with accelerated luteinization of the theca interna and later after rupture of the large portion of the granulosa remaining in place According to Fevold[85] the luteotropic hormone stimulates the secretion of progesterone from the luteal tissue laid down by the luteinizing hormone

As the theca rapidly proceeds with luteinization and the production of progesterone gradually increases the ovum still in the prophase of the *primary oocyte* stage[70] undergoes its final preparation for release and fertilization i e the reduction of its chromosome number from the diploid 48 * to the haploid 24 This is accomplished in the mammals that have been studied in the course of two successive divisions uninterrupted by a resting phase the so called *maturation* divisions

One of these is reductional—hence its designation *miotic* (Greek *meion* smaller less)—and leads to a halving of the chromosome number The other is equational thus resembling an ordinary mitosis That in the human as in all mammals investigated with the exception of the dog the *reductional division* occurs first while the egg is still in the ovary[42] is attested to by the recovery by Allen *et al*[8] from a normal 10 mm ovarian follicle of an oocyte in which 22 peripherally located chromosomes in metaphase were counted Evidence that this was the metaphase of the *second* division is provided by the presence of one *polar body* still associated with the ovum

* Tjio and Levan [72] have recently reported that by utilizing a new technique they found the diploid number of chromosomes in the human to be actually *46 instead* of 48

that is reduction to the haploid number of chromosomes must have already taken place during the *first* division which resulted in the formation of the *first* polar body Moreover Pincus and Saunders[1] counted the haploid number (24 dyads) of chromosomes not only in the oocyte but also in the one polar body of a human ovarian egg which they considered had also attained the metaphase of the *second* division in response to activation *in vitro* by hypertonic solution Hence here again reduction of the chromosomes to the haploid number must have occurred during the *first* division

In this first (miotic) division it is believed on the basis of animal observations—for but few human ova have been found in maturation [1] —that the maternal and paternal members of each pair of homologous chromosomes come together in *synapsis* forming as it were 24 double chromosomes[70] These locate themselves on the spindle at metaphase[84] In the anaphase[180] the two members of each synaptic pair separate one set going to each pole of the spindle Thus when cytoplasmic cleavage occurs 24 chromosomes remain in the egg (now a *secondary* oocyte) while the other 24 pass into the first polar body The redistribution of chromosomes that takes place here accounts for the various combinations of grand maternal and grand paternal characteristics contributed by each gamete to the zygote The first polar body may itself divide before the egg is fertilized or before the second polar body is released [234]

Without a period of nuclear reconstruction there now rapidly follows the second (equational) division in which at metaphase the 24 chromosomes (dyads) repose on the spindle According to Allen and his associates[8] it is at this stage that ovulation occurs in most mammals That this is true also of the human is evidenced by the fact that the three *youngest apparently normal human unfertilized tubal ova* which have been described are also in the metaphase of the second maturation division[9,10] In most mammals the completion of the second maturation i e anaphase and cytoplasmic cleavage with formation of the second polar body is believed to take place only if the spermatozoon enters the egg[42]

Moricard[1] states that the ovule remains in the stage of primary oocyte until several hours preceding ovulation A few hours before rupture when the ovum is in the metaphase of the first maturation division the follicle has attained its full size In the human this is usually stated as 10 15 mm in diameter[73,9] Watzka[91] however in his excellent monograph on the ovary cites figures for the size of the ripe follicle ranging between 10 and 22 mm That the range may actually be wider is indicated by the recovery in our laboratory of a human oocyte judged to be 0 to 1 day preovulatory from a 28 mm graafian follicle![109]

When it is recalled that the primordial follicle reaches a diameter of only about 30 μ [11] a preovulatory follicle measuring 15 mm (15 000 μ) represents a 500 fold increase!

The ovum itself does not undergo such a spectacular augmentation From a diameter of about 20 μ which the primordial ovum attains before it is enclosed in nurse cells (v s [18]) the vitellus in the preovulatory graafian follicle reaches a median size of the order of about 100 μ[8,1,7] i e a fivefold increase When however the width of the encircling *zona pellucida* is taken into account the preovulatory ovum may reach a diameter of 117 145 μ[8,71] i e up to about a sevenfold increase

From these calculations of the size of ova in large follicles as well as from the sizes of the earliest normal tubal ova recovered[9,10] it may be said that at the time of ovulation the diameter of the human egg averages about 130 μ the vitellus being of the order of 100 μ and the zona pellucida 15 μ Watzka [9] states that in the ripe egg the nucleus is 20 27 μ while the nucleolus measures about 6 μ

A concise description of the main features of ova and follicles in early and mature stages is given by Shaw [3] For a more detailed account of the cytology of ova from preovulatory follicles the reader may consult the microsurgical studies of Duryee [1] as well as the reports of observations made by Shettles[233] with phase contrast microscopy Watzka [98] in addition to a complete description of the ripe egg presents a scholarly review of the microscopic characteristics of both ova and follicles at all stages of development up to the time of follicular rupture

(d) OVULATION

As will be described later the follicle soon after luteinization ruptures and the mature ovum is

Watzka [90] states that the *zona pellucida* or *oolemma* regarded by some as a secretion of the granulosal cells of the corona radiata makes its appearance at the same time as does the *liquor folliculi* In follicles of 1 mm diameter according to Watzka the ovum is already surrounded by a well-defined zona pellucida measuring 2 5 μ By the time the follicles have attained preovulatory size the zonae pellucidae of the contained ova range from 12 to 18 μ in thickness More recent microsurgical investigations of Duryee[71] likewise on human ova from large follicles led this author to report that the zona pellucida instead of having a definite membranous or fibrous structure as had been generally supposed consists of a viscous jelly supporting the corona cells In the course of this study Duryee also made the interesting observation that so-called coronal canals or outpocketings of the vitelline membrane traverse the zona pellucida to connect up with the cells of the corona radiata He regards these canals as functional units which transport nutritive materials to the egg

floated out in the follicular fluid Sadly the hormonal as well as other possible factors of actual dehiscence of the thinned follicular wall are still unknown Sturgis and Taymor (to be published in *Fertility and Sterility*) have found a notable increase in urinary excretion of LH in close temporal relationship to the low point on the basal temperature curve The significance of this is still abeyant

With rupture the process of luteinization which had started in the theca interna during the third stage of follicular maturation is now continued in the theca and what remains of the granulosa [96] More progesterone as well as estrogen is secreted As the supply of estrogen rises beyond a critical level the hypophyseal secretion of FSH diminishes Thus LH together with the still moot LtH if present in humans acquires ascendancy

Perhaps as Astwood [13] *postulated for* the rat to LtH we should attribute the major part in development and sustenance of the corpus luteum in the human during the approximate 14 days of luteal function The evoker of LtH in the hypophysis is obscure Astwood by 1941 had found no evidence of cyclic variations in the amount produced It would seem to act only after LH has produced luteinization and its effect Astwood proposes would seem to diminish during the second part of postovulatory pseudopregnancy when the corpus luteum gradually regresses and titers of urinary pregnanediol go down However if LtH is administered to rodents the corpus luteum can be made to persist and thus to prolong postovulatory pseudopregnancy [69] This declining gland is revived normally only by *chorionic hormone* secreted by the trophoblast of the differentiated conceptus

(e) FOLLICLE ATRESIA

We have followed the human graafian follicle through its various stages of development A similar process is seen in several other follicles that have been sensitized to FSH by estrogen They reach the degree of maturity at which they are ready to start luteinization and indeed some may even do this (Hisaw s third stage of development) But here they usually fail because of a delicate species quality In most instances in the human female there seems to be merely enough of the synergistic hormones LH and FSH to evoke in only the most receptive follicle effective amounts of progesterone and increased estrogen This most sensitive follicle apparently takes most of the supply and its own resultant endocrine activity diminishes the hypophyseal product (FSH) below the reactive threshold of the other follicles An alternative hypothesis is that appreciable amounts of progesterone produced by the dominant follicle desensitize the other follicles to the FSH and LH present

There is no effective answer as to why certain of these follicles become atretic [97] However it seems quite plausible that the immediate cause might be incomplete differentiation of the theca interna It is known that many more follicles are capable of being brought to ovulation than normally occurs [1 0] This can be induced in several species by injecting gonadotropic preparations [184 1 6 7 97] As a rule in the human only one follicle ruptures The other competing follicles regress Yet there is evidence of more than one ovulation occurring in the human at the same time as is possibly the case in instances of dizygotic multiple pregnancies On the other hand there is the rare possibility that such may result from fertilization of both of two blastomeres from a single *ovum cleaving parthenogenetically* [1] *That* rupture in more than one follicle may occur is evidenced by the interesting case reported by Allen *et al* [9] in 1930 in which twin ova which appeared normal and similar were isolated one from each oviduct These authors remarked that Each ovary contained a corpus luteum with a prominent recent rupture point Shaw [31] likewise mentioned three instances in each of which two corpora lutea of similar age were present At the Free Hospital for Women we have observed at least one such case indicating that ovulation involving more than one graafian follicle had occurred

3 MEDIATION OF NEUROHUMORAL MECHANISM IN CONTROL OF FOLLICULAR DEVELOPMENT AND OVULATION BY ANTERIOR PITUITARY GLAND

Until about 1940 the reciprocal interplay between hormones from the follicle and corpus luteum on the one hand and the gonadotropins from the hypophysis on the other together with the cyclic nature of their push and pull especially in the adult woman indicated that all these factors exercise a purely endocrine function that through the blood stream the individual hormones reach their secreting target cells However in the rabbit and some other mammals regularly as in the ferret and cat commonly coitus precipitates ovulation Indeed mere sexual excitement of the imprisoned doe by a proximate but separately caged buck may evoke follicular rupture

In 1936 Marshall and Verney reported that in a rabbit doe in heat stimulation of the central nervous system by a strong electric current either through the brain or the lumbo sacral part of the cord would induce ovulation The demonstration in 1947 by Sawyer Markee and Hollinshead [219] that the ovulation which would normally follow coitus

in the rabbit was blocked by the sympatholytic drug Dibenamine if given within three minutes of the act lent support to their hypothesis that ovulation in the rabbit is dependent upon a humoral pathway *of an adrenergic nature*

Two years later Everett Sawyer and Markee[80] found that even in the rat which does not ovulate by virtue of coital excitation ovulation could be prevented by such adrenalin antagonists as Dibenamine or atropine if either were given at that time in proestrus when the follicles were merely approaching maturation Moreover on the basis of their findings these investigators were actually able to time the exact occurrence of the so called neurohumoral stimulation of the adenohypophysis which they now postulated as causing ovulation that is 10 12 hours preceding this event

The theory of a neurohumoral mechanism to account for such phenomena had already been suggested by the discovery in 1940 by Westman and Jacobsohn[293] that although in the rat grafts of anterior hypophyseal tissue in the anterior chamber of the eye would grow to even more than twice the size of the normal gland with histological similarity to *it yet no gonadotropic activity could be found in* them In 1952 Harris and Jacobsohn[108] demonstrated that this function could prevail only if the transplant were placed beneath the hypothalamus and revascularized by capillaries of portal vessels arising in the median eminence of the brain

Whereas Zuckerman[308] has presented evidence which questions this concept Donovan and Harris[67] in similar experiments have reported results which confirm the role of the pituitary stalk in this connection These authors in their comprehensive re*view of pertinent work make clear the concept that* neural components of the hypothalamus reacting to afferent nervous stimuli originating elsewhere in the body as well as to estrogen or progesterone may by virtue of this effect produce substances which upon entering the portal system of the hypophysis reach and influence the gonadotropin producing cells within the anterior pituitary gland

Figure 2 *reproduced from the paper by Cleghorn* and Graham[43] depicts the course of the blood from highly vascularized nuclei of the hypothalamus into the thin walled capillaries of the hypophysis by which route the hypothetical neural humors reach the gonadotropin producing cells

Thus through the thalamus may the environment and even the emotions of woman interfere with her normal cyclic ovulation and menstruation This is not to imply that human ovulation is subject to rab bit like sexuality and certainly not even to suggest that if the human ovum is once freed its treatment by the oviducts and uterus can be affected by emotional attitudes The inviolability of the corpus luteum which conditions these structures and particularly the autonomy of the cytotrophoblast which takes over from the corpus luteum is not to be underestimated

II THE OVULATORY PROCESS

In 1672 Regnier de Graaf[60] described what we now refer to as the *graafian follicle* De Graaf however did not understand the true significance of these fluid filled vesicles but thought as had van Horne[2 3] before him that they were ova It was not until 1827 with von Baer s[15] discovery of the mammalian ovum in the follicle of a dog that the true relation between ova and follicles was properly understood Another milestone in our knowledge in this connection was the isolation by Corner[47] in 1923 of the first living unfertilized tubal egg of a primate (macaque monkey)

A Direct Observation of Ovulation

In 1928 Walton and Hammond[287] *observed follicu*lar rupture which they had caused in the rabbit and recovered the ovum from the follicular fluid In the human however ovulation was not described until 1951 when Decker[58] reported having seen follicles during rupture His impression was that Ovulation is not always an abrupt complete spilling of the contents The follicle dehiscence may be minute and hours or days may be required for the contents to escape

More recently detailed observations of the rupture *of graafian follicles in the intact immature rat stimu*lated by gonadotropin have been reported by Blandau[24] He remarked that as a rule the ovulatory process in a large follicle was of an explosive nature while appearing as a gentle ooze from follicles of intermediate size It was noted that the ovulatory follicles usually bulge forth from the cortical surface as a result of the thinning of the germinal layer and *of their own thecal layer This gives the appearance* of moderate distention while over the surface a characteristic vascular pattern is discernible Impending rupture is heralded by the development of the *macula pellucida* or *stigma* at the apex of the follicle The superficial germinal epithelium here can be observed to break up into clumps at the start of the disruption of the surface layers which is then followed by the escape of the follicular fluid The ovum surrounded by the corona radiata of granulosa cells within the cumulus is floated out in the fluid along with clumps of granulosa

B Theories To Explain the Local Mechanism of Follicular Rupture

In the first edition of *Sex and Internal Secretions* published in 1932 Carl G Hartman[10] devoted eleven pages to a discussion of the various theories that had up to then been postulated to explain the local mechanism of follicular rupture After citing evidence *pro* and *contra* such causes for rise in intrafollicular pressure as (a) secretion of *liquor folliculi* (b) increased hyperemia and blood pressure (J C Clarke) (c) swelling of theca interna cells (Waldeyer) (d) *nerve muscle factors (muscular action* in the follicle wall in the ovarian cortex or even in the tubal fimbria Rouget Pflüger the Guttmachers Grosser) *as well as (e) the effect of* proteolytic enzymes hypothecated by Schochet Hartman concluded

Enough has been said to render the idea reasonable that mechanical factors alone cannot bring about ovulation but that normally the follicle undergoes growth changes within the wall that are essential to the rupture

In 1937 J T Smith[231] made the interesting observation that after the injection of pregnancy urine there was an increase in osmotic pressure in the ovarian follicles of the rabbit In the following year this same author[52] attributed the rise of intrafollicular pressure directly to the activity of the so-called Call and Exner bodies which he believed contributed possibly glycogen or a sugar to the follicular fluid

Strassmann,[2 6] on the other hand in 1941 after listing and discarding all previous hypotheses as to the local mechanism of ovulation presented his own explanation based on eighteen years of microscopic research on the ovaries of man and animals Strassmann concluded that the local mechanism of ovulation could be explained by the eccentric growth of the theca interna (the so-called theca interna cone) which he had observed in his material According to Strassmann this cone is in the form of a triangular shaped wedge which, growing to the ovarian surface by what he postulated to be a tropism functions as a pathmaker for the ascent of the growing follicle This author regards the theca interna cone as an integral part of the mechanism of ovulation In 1950 Schwarz and Young[2*] reported their confirmation of Strassmann s observations

About ten years ago Kraus[155] having tested the various hypotheses that had been advanced in connection with the mechanism of follicular rupture concluded that in the rabbit neither internal pressure local enzyme action muscular forces nor any action of the fimbria of the oviduct was involved Kraus therefore postulated that there occurred a morphologic change in the stigma the cause and nature of which were unknown. This would seem to be *in perfect harmony with the conclusion stated by* Hartman[10] 15 years previously (v.s) However more recently an interesting suggestion has been offered by Barjaktarovitch[1] in this connection To quote from an English abstract of his work

It is believed that when the intrafollicular tension rises to a maximum as a result of the action of pituitary gonadotrophins the nerves of the theca are stimulated and this results *in stimulation of the pituitary* The latter produces not only LH but also the oxytocic hormone of the posterior lobe which causes contraction of the theca Under the influences of these contractions—similar to those of the gravid uterus—the follicle bursts the connections between the corona radiata and its base are ruptured and the ovocyte is expelled

In the abstract cited this author did not include any experimental basis for his concept nor have we encountered any such evidence elsewhere in the literature *In animal experiments (frog and hen)* Kraus[135] had failed to observe any effect of oxytocin or other smooth muscle stimulants on ovulation

C Life Span of Ovulation

The age period during which ovulation takes place in the human is defined at one end by the *menarche* and at the other by the *menopause* These are phases in life respectively associated with the appearance and disappearance of menstruation but this visible sign is not always indicative of ovulation although the latter begins sometime during the *menarche and* ceases around the time of the menopause While the only exterior sign conclusive of a previous ovulation is the establishment of a pregnancy *evidence* of strong progesterone effect is generally accepted as decisive Although—*conception failing*—an apparently normal menstrual flow usually follows ovulation at an interval of about 14 days such catamenia cannot be considered *proof of the actual occurrence of* follicular rupture

The report by Escomel[76] from Lima Peru of conception in a child a bit under five years of age who was delivered by cesarean section at term of a normal infant records the earliest pregnancy Section of her ovary revealed full maturity However this should not be regarded as a normal case for certain signs in this *youngster indicated* that she was afflicted with a syndrome previously described by Albright and his associates[2 3]

It should be pointed out that *in view of the relatively* few pregnancies that occur from coitus during *early menarche* as well as the fact that as puberty

merges into adolescence menstrual flow is usually for a variable time irregular and dissimilar the first ovulation in most girls is thought to follow the onset of menstruation only after some months even years [90]

The age at which menstruation begins has been the subject of countless publications Although race climate and constitution have for years generally been regarded as fundamental in this connection Fluhmann [89] in a critical survey of the available data discounts the importance of these factors While he points out that the question is by no means settled the evidence compiled by him warrants his dictum that the most significant [factors] appear to be proper nutrition and healthy living conditions Supporting this viewpoint is the table he presents (p 63) in which are listed the mean ages at menarche of white American girls as computed by various authors between the years 1884 and 1948 The values range from 14 23 years (1884) to 12 90 years (1948) the downward trend as noted above being ascribed by Fluhmann to improved economic and health conditions At present Fluhmann states the range of start of menarche among the great majority of American girls is from eleven to sixteen *years with a mode at about thirteen years* *After* remarking that American girls appear to have an earlier menarche than those of any other country in the world Fluhmann goes so far as to conclude his discussion by observing It seems clear that the age of the menarche is an index of the degree of civilization and well being of the people of all countries

The upper age limit at which a woman may give birth recently studied by Newell and Rock [166] is stated by them to be 52 years These authors concluded from their survey that parturition over this age had not been proved Anderson [10] has since presented a well authenticated case of an abortion in a woman age 56

One may conclude that the ovulatory process usually begins in the early teens and may continue to about age 50 with individual variations at either end of the span

D Anovulatory Episodes Within the Ovulatory Life Span

Moreover even within the temporal limits of ovulatory function in a given woman there may be fluctuations of activity As summarized previously by one of us (J R) [194]

Unfortunately the cycle of ovulation is frequently interrupted sometimes as often as several times a year in many females who consider themselves "regular" because the ovulatory mechanism is subject to various environmental as well as intrinsic functional disturbances This commonly occurs also in recent mothers during the first two or three months after weaning as well as in females during adolescence and after the age of about thirty eight An egg is not always ripened and released on time

How to determine the incidence of anovulatory menstruation among normal women is as Siegler [213] has pointed out fraught with insurmountable difficulties He remarks

No valid estimate as to the frequency of the anovulatory cycle in women is possible The problem has chiefly been studied in the relatively small group of women under investigation for sterility and of these in many instances only a single biopsy was taken for diagnosis It is now generally accepted that anovulation is more often sporadic than permanent and that a progestational endometrium in one cycle does not exclude the possibility of an anovulatory endometrium in another No final information can be obtained on these points but if 1000 women between 20 and 40 years were to be examined by a biopsy every month for a year we would have a far more accurate picture of the regularity and frequency of ovulation than at present The significance of such *studies is increased if the patients* are studied by age periods There is no doubt that anovulatory menstruation occurs more often after 35 than in patients of 20 to 30 and also more often in patients between puberty and age 20

As regards the incidence of anovulatory menstruation in women undergoing treatment for sterility Siegler [213] after compiling the results of several authors found the values to range between 1 and 30 per cent In his own series of 953 patients investigated for involuntary infertility between the years 1946 and 1950 the frequency of anovulatory menstruation was 5 77 per cent At the Fertility and Endocrine Clinic of the Free Hospital for Women Rock Bartlett and Matson [196] having performed endometrial biopsies on 392 women in the course of routine sterility work up concluded that about 4 per cent may be considered habitually anovulatory while 9 per cent showed evidence of at least occasional anovulatory menstruation

Concerning the time of resumption of ovulation after pregnancy Sharman [230] in 1951 summarized the results of previous investigators and reported his own studies after delivery abortion and operation for ectopic pregnancy In his series of nearly 400 normal postpartum women he found the earliest onset of ovulation on about the 42nd day after delivery while the latest anovulatory specimen was obtained as late as the 28th week postpartum

In lactating women the first ovulatory biopsy was detected at the 13th week In two instances proges

tational endometria were observed at the 49th day from lactating women who did not menstruate until 10 weeks later

Among the patients in the postabortum group the earliest progestational specimens were obtained 22 days after the abortion and the latest anovular biopsy in the 7th week

Ovulation in the postectopic series was found to have taken place as early as 19 days following operation while failure of ovulation was noted as late as 16 weeks postoperatively

E Pattern of Ovulation

In a given cycle ovulation usually occurs in only one ovary in the human although Dickinson [65] on the basis of repeated pelvic examinations reported that both ovaries enlarge and become tender to pressure at the time of ovulation That rupture in more than one follicle may be the cause of dizygotic multiple pregnancies has been confirmed by such observations as those of Allen and his associates Shaw [231] and in at least one instance at the Free Hospital for Women

As regards the *position* or *site* of ovulation in the ovary van Wagenen and Morse [251] from their studies on the macaque monkey reported that No significant difference in choice between dorsal and ventral surfaces appeared There was a tendency for ovulation to take place toward the tubal end of the ovary (more than twice as often) and equally as often ovulation occurred toward the free margin rather than the hilus of the ovary

Nor does ovulation occur with any particular pattern of frequency as far as any one ovary is concerned In 1936 Morse and van Wagenen [1 1] having confirmed the observations previously recorded by Hartman [110] stated

> in the *Macacus rhesus* although the ovaries as in other animals must be subjected to the same hormonal influences there is no rule for the sequence or for the alternation of ovulatory function Indeed as was shown in one animal in which of a total of 7 observations the corpus luteum was found upon the right side in six ovulatory activity may be confined to one ovary although the other organ is present and as far as can be determined anatomically normal

From numerous observations of surgeons and pathologists we know this to be true also of women

It is pertinent to point out here that in three unilaterally ovariectomized monkeys van Wagenen [2] found ovulation to take place in the remaining ovary in 14 out of 20 cycles It is common knowledge that in the human female also if one ovary is absent by virtue of agenesis destruction or removal the remaining gonad by itself functions with the woman s characteristic rhythm

F Time of Ovulation Criteria and Methods of Detection of Follicular Rupture

Because ovulation cannot be viewed directly with ease and frequency one must rely on indirect methods *to detect its occurrence* As will be noted later many of these may be misleading and it is little wonder that throughout the centuries there has been much confusion concerning the time of human ovulation [29] Furthermore one must depend in many instances on animal data which are not always *transferable to man*

The time of occurrence of ovulation in women has great biological and clinical significance This knowledge is of inestimable value in problems of infertility in diagnosis and treatment of disorders of menstruation in handling of some disturbances of pregnancy as well as for the suppression of fertility

In the classic volume published by Hartman [112] in 1936 are reviewed the data then available regarding the time of ovulation The historic account of Bryant [9] which surveys the theories of ovulation from earliest times up to the year 1939 is also of interest In 1941 one of us (J R) [193] attempted to review briefly the status of the problem up to that date Since then numerous writers have published articles on this subject among them Pommerenke [1 5] in 1944 Bergman [2] and also Sturgis and Pommerenke [265] in 1950 and more recently Brown [3]

Methods for detecting ovulation may be divided into those which furnish positive proof of ovulation having occurred but leave some question as to the exact time of this event and those which provide presumptive findings that, if utilized alone may *give questionable results* but become more significant if they co-exist with others A *limited time relationship* among these latter would be difficult to explain on the probability of chance alone

1 Methods Giving Positive Proof of Ovulation

(a) THE ESTABLISHMENT OF A PREGNANCY

Approximate dating of ovulation may be *made* in instances of isolated coitus that result in pregnancy [2] [5] as well as in cases of women submitting to successful artificial insemination [133 1] These controlled exposures serve to prove that ovulation took place and one can measure the preovulatory phase of the specific cycle by this method The early embryos studied by Hertig Rock and Adams [119] furnish additional significant information as to the time of ovulation (v i)

(b) DIRECT OBSERVATION (CULDOSCOPY CULDOTOMY AND LAPAROTOMY)

Visualization of a fresh corpus luteum by either culdoscopy culdotomy or at laparotomy witnesses to the fact of ovulation [58, 6, 9, 171] at least until some one proves that the yellow body may form without the prior occurrence of follicular rupture Likewise a protruding follicle in the absence of a recent corpus luteum may indicate that ovulation is immi nent

Histologic dating of an excised corpus luteum fixes ovulation time with fair precision Corner Hart man and Bartelmez [19] accurately dated corpora lutea in the rhesus monkey These investigators estab lished criteria that were later applied to human ma terial by Brewer and Jones [26]

Allen Pratt Newell and Bland [9] recovered unfer tilized ova from human oviducts and correlating these specimens with studies of the associated cor pora lutea concluded that ovulation occurred on about the 14th day of a 28 day cycle Rock and Hertig [198, 9] likewise having collected human unfer tilized ova in addition to early embryos compared the estimated ages of these with the respective endo metrial histology as well as with the corpus luteum in each case Their results indicate that the postovu latory phase is nearly constant namely about 14 days before the first day of the next expected men strual period An illuminating critique of the inter pretation of these results as well as a comprehen sive and discerning discussion of other available data on the length of the luteal phase has more recently been presented by Hartman [113]

2 PRESUMPTIVE METHODS FOR DETERMINING THE DAY OF OVULATION

(a) ENDOMETRIAL BIOPSY

It is usually stated that Kundrat and Engelmann [138] in 1873 were the first to study the changes in the human endometrium during the menstrual cycle However these studies as well as those of Wil liams [298, 300] in 1874 75 were all carried on with post mortem material It was not until 1908 that a thorough study of the human endometrium in living subjects was correlated with menstrual data by Hitschmann and Adler [121] Later Schroder [2 1] in a large series of women correlated the endometrial findings not only with cycle day but also with ovarian morphology In 1937 the observations of Rock and Bartlett [195] on endometrial biopsies in relation to menstrual dates led these authors to conclude that these biopsies afforded a moderately reliable indication of ovulation time Noyes Hertig and Rock [170] in 1950 extended this technique and their criteria are now widely accepted as a fairly ac curate method for determining the duration of pro gesterone effect on the endometrium and thus pro vide an indication of the date of the immediately *preceding ovulation A rapid method for detecting* the progestational phase has more recently been pre sented by Sabin and Latour [215] Endometrial biopsies have been correlated with several other indices of ovulation time [151, 104, 17, 3, 1, 8]

(b) BASAL BODY (WAKING) TEMPERATURE

As far as we are aware the earliest recorded ob servation of fluctuations in body temperature during the human menstrual cycle was that of Squire [37] who in 1868 published the talk which he had pre sented before the Obstetrical Society of London in the preceding year The splendid reviews of D S Barton [16] in 1940 and of A Palmer [172] in 1949 in clude comprehensive historical accounts of the perti nent investigations in the intervening years As early as 1876 Mary Putnam Jacobi [1 7] on the basis of numerous recordings of body temperature at dif ferent times of the day of different parts of the *body and in many women came to the conclusion* that there was a higher body temperature before menstruation than in the first part of the cycle In 1904 Van de Velde [276] presented his classic essay on the subject In it he pointed out the advantages of taking temperatures early in the morning upon awakening (leading to the adoption of what the English call waking i e basal temperatures)

More than 30 years later Rubenstein and Linds ley [212] in a preliminary note reported on their ob servations correlating changes in basal body tem perature with the cytology of vaginal smears Also in 1937 Rubenstein [07] published a longer article in which he called attention to the relationship between the low point on the temperature curve and the so called ovulative vaginal smear the high point on the other hand he associated with the premen strual smear Rubenstein [08, 0] continued his investi gations during the next few years Also Zuck [3 5] in 1938 M Barton and Wiesner [17] in 1945 as well as *many others have found* that the waking tempera tures in women show fluctuations which reflect criti cal endocrine changes of the ovarian cycle Nie burgs [16] in particular has analyzed the hormonal aspects of temperature variations

In 1943 Greulich Morris and Black [1 0] attempted to correlate thermal changes with morphological find ings in the ovaries Buxton and Engle [39] several years later carried on similar investigations and in addi tion reported their observations on the endometrium

In 1944 Tompkins [273] stressed the clinical appli cability of the basal temperature method and devised a convenient form for the patient s use in recording

her daily temperature When taken under basal conditions preferably on awakening and before muscular activity at about the same time each day these graphs show a relatively lower level during the preovulatory than during the postovulatory phase The initial rise of at least a fraction of a degree is usually maintained until at or near the onset of menstruation A characteristic so called *biphasic* curve is obtained in the patient who ovulates In the anovulatory cycle on the other hand there is no characteristic rise thus giving a curve which is designated as *monophasic* As pointed out by M E Davis[53] these temperatures may be obtained with equal reliability orally vaginally or rectally However since many patients have lost the thermometer in the bladder by mistaking the urethra for the vagina and some women find it inconvenient to insert it rectally mouth temperatures are preferable

Frequently there is noted a sharp drop in temperature just preceding the rise This latter is sustained for approximately 14 ± 2 days before the onset of catamenia The temporary decrease approximately synchronous with the increase in urinary estrogen is considered indicative of the ovulation phase

The accuracy and reliability of a temperature shift for pointing out the time of ovulation has been questioned by many However the day of the low point from which the temperature thereafter rises to stay for 14 ± 2 days seems to be acceptable as the day on or near which the corpus luteum begins to function, and therefore to approximate ovulation time

To our knowledge the earliest suggestion of the relation between the sudden rise in temperature and the functioning of the corpus luteum was made in 1928 by Van de Velde[278] It is also of interest in this connection that as far back as 1904 this author[276] had reported that in a menopausal woman the temperature curve could be re established through the influence of ovarian tablets In 1938 Rubenstein[98] stated the temperature raising effect of progesterone may be demonstrated at will either clinically or experimentally However he cited no references to such investigations Later in the same year R Palmer[173] on the basis of preliminary studies also proposed that the thermal rise was due to the action of progesterone and shortly afterwards this author in collaboration with Devillers[174] presented indubitable experimental evidence in support of this concept These findings were later confirmed by other workers[13 8 3 84]

By careful comparison of ovarian morphology i e studies of follicles and early corpora lutea observed at laparotomy with temperature graphs kept during the operative cycle Buxton and Engle[3] found a difference of as much as four days between the rise in temperature and the age of the corpus luteum Buxton[37] therefore later proposed that ovulation could be considered as occurring on or within two days before or after the day the temperature first rises

In our experience if the elevation is sustained for more than 18 days pregnancy may be suspected and if for a period of 20 days or more then the diagnosis of pregnancy becomes fairly certain Lest one be misled into oversimplification of the thermal sign of ovulation we present six temperature charts each kept during a conceptive cycle (Figs 3 and 4)

(c) EXFOLIATIVE CYTOLOGY

In 1917 Stockard and Papanicolaou[65] observed cyclic histologic variations in the vagina uterus and ovary in the guinea pig Several years later Long and Evans[145] as well as Allen[5] applied these findings to the measurement of endocrine changes In 1923 Corner[47] first studied the vaginal cycle of a primate *Macacus rhesus* Finally Papanicolaou[175] in 1933 reported his ability to ascertain the time of ovulation within two or three days of some although not all women by determining the degree of cornification of squamous cells in vaginal smears He concluded that ovulation occurred most often between days 12 and 13 of the cycle with a range of days 7 through 17 E Shorr[235-8] has developed simpler techniques for staining vaginal smears as has also Mack[47]

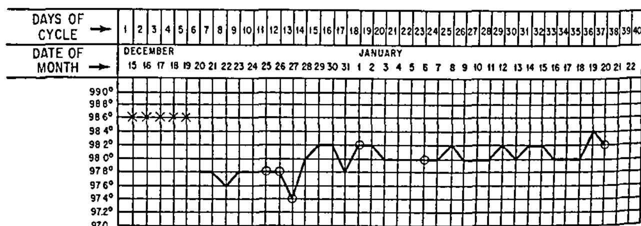
The use of vaginal cytology to detect ovulation has been reviewed by many authors[57 175 56 9 92] The vaginal smear method requires daily collection of specimens throughout the cycle as well as special staining Although the smears are read rapidly considerable training and experience are required of the interpreter Despite the ability to recognize a change in the quality of cells in the desquamates pinpointing of ovulation is practically impossible by this method In addition any vaginal irritation interferes with the interpretation as this may produce cornification independently of hormonal response The clinical practicability of this technique is limited to determining the occurrence of ovulation, but not the exact time The mere 70 per cent correlation of vaginal smears with ovulatory cycles by Goldhar Crody and Masters[9] corroborates this

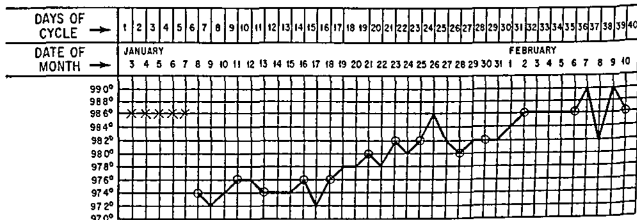
Other desquamates While Romberg[98] has reported similar treatment of endometrial samples obtained with a fine uterine aspirator there does not seem to be much advantage in this procedure If one is to introduce a tubule into the cavity of the corpus one can with no more effort and not much more discomfort to the patient accomplish an endometrial biopsy which is far more accurate not only in detecting but also in dating ovulation

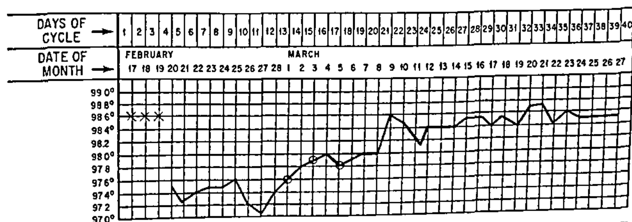
It has been shown that epithelial cells in the urinary sediment increase in number at ovulation time [69] Furthermore during each cycle these follow similar changes in pattern as do the cells from the vagina [61] This method has been suggested as a substitute for the vaginal smear a voided or a catheterized specimen affording equal accuracy Urine collection although simple may be cumbersome and by means of a cotton swab or an aspiratory pipette the patient can almost as easily obtain the vaginal desquamate and place the slide promptly in the proper fixative provided

(d) VARIATIONS IN HORMONE LEVELS DURING THE MENSTRUAL CYCLE

(1) *Estrogen* Estrogen in blood serum as assayed by a modified Allen Doisy test was reported by



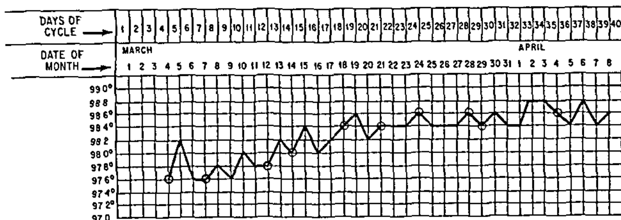


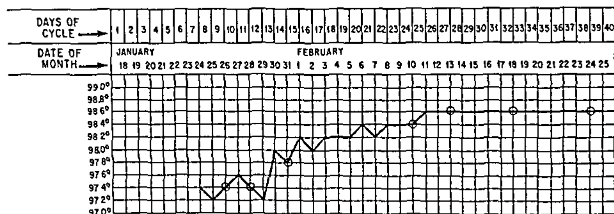


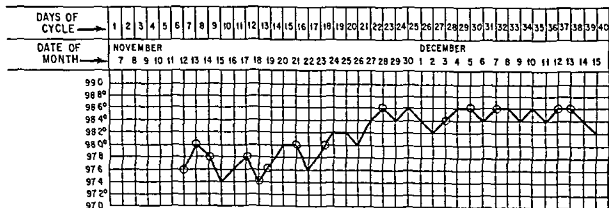
FIGS 3 AND 4 TEMPERATURE CHARTS OF SIX NORMAL WOMEN

Fluhmann[58] in 1934 to show a high point at about midcycle with a secondary rise at the time of catamenia. Cyclical fluctuations of urinary excretion of estrogen have been noted by others.[10, 24, 30, 101, 25] Parallel excretion of estrogen in the urine and feces has also been studied.[2, 1] Since ovarian follicular development appears to follow the estrogen curve with the peak at or near the time of ovulation, such assays have been used as a means of detecting ovulation time. The temporal position of this peak according to Smith and Smith[49] lends support to the concept of the relative constancy of the luteal phase of the human menstrual cycle as compared to the follicular phase. Furthermore, the shift in the estrone/estriol ratio at midcycle has been regarded as an index of luteal function and hence of ovulation time.[50]

A comprehensive discussion of the estrogens in







EACH GRAPH HAVING BEEN RECORDED DURING A CONCEPTIVE CYCLE O (CIRCLE) = COITUS

relation to the human menstrual cycle was published in 1952 by Smith and Smith *

(ii) *Pregnanediol excretion* The clinical significance of pregnanediol excretion has recently been reviewed by Rogers [93] In the human as in the rabbit pregnanediol has been established as the urinary metabolite of progesterone [28] Furthermore it can now be measured by relatively simple methods The finding of appreciable amounts of pregnanediol in the urine indicates the presence of a corpus luteum from which one may assume that ovulation has occurred Assays of pregnanediol and more recently of blood progesterone [93] indicate a rise in these levels at about ovulation time or just preceding it with pregnanediol showing a peak 3 5 days before menses [25]

(iii) *Gonadotropin levels* The peak of excretion of gonadotropins from the anterior pituitary gland is believed to occur at or near the time of ovulation D Amour [50 51] stated that this point of highest excretion is a more reliable index of ovulation time than can be deduced from vaginal smears or basal body temperature graphs In the studies reported this peak occurred between the 12th and 16th days before the onset of the succeeding catamenia

An application of the gonadotropin assay is the *Farris* or *rat hyperemia test* [83 4] This utilizes the hyperemic response in the ovaries of an immature rat to urine containing the gonadotropic substance Farris reports the reaction to be most intense at the time of ovulation There is a variance of opinion as to the validity and accuracy of this test [144] While it appears to give valuable and consistent results in the originator s laboratory other workers report failure to confirm them At best the practicability of this method is questionable unless one has available a large colony of Wistar rats [126]

(e) CERVICAL MUCUS

The *cervix secretes mucus* which changes in appearance and properties throughout the menstrual cycle At ovulation time it becomes thinner clearer more profuse and more receptive to spermatozoa The cervical mucus at other times particularly after ovulation appears thick scant cellular and occasionally even gelatinous

In his excellent historical review Abarbanel [1] credits W Tyler Smith with the first recognition in 1855 of the cyclic variations in the qualities of cervical mucus Eleven years later J Marion Sims [246] elaborated on this finding His observations constitute the basis for the so called *Sims Huhner test* now a routine method in the investigation of conceptive failure

In 1933 Sequy and his co workers [226 7] from their study of these changes concluded that ovulation occurred between days 10 and 15 in cycles of normal length Lamar and his associates [140] on the basis of similar investigations placed ovulation between the 9th and 19th days of the cycle Extensive studies on the relation of cervical mucus to the cycle have been carried on in Pommerenke s laboratory [83 189]

We have not found this method (i e by physical criteria) to be precise for dating ovulation although it usually is adequate for identification of the ovulation phase This is due to the fact that the listed properties in a given patient may be minor and either protracted or of very short duration Therefore we believe the dependability of any postcoital examination of cervical mucus as a test of insemination must be evaluated with careful reference to the amount viscosity and cell content of the fluid extracted

In addition to cyclical physical changes in cervical mucus Pommerenke and Viergiver [189] have also reported a shift in the concentration of glucose or related compounds at midcycle

At or near the time of ovulation constituents of the cervical mucus when dried have been observed to crystallize and form characteristic patterns † resembling palm leaves or fern [176 7 214 205 40] Furthermore at this time the mucus displays a decreased cohesiveness which permits the stretching of it into long threads of 10 20 cm or more before it breaks [44 5] The former characteristic is termed *arborization* and the latter *Spinnbarkeit*

Stein and Cohen [260] state that in the absence of estrogen therapy maintenance in cervical mucus of sperm motility for 24 hours or more after coitus indicates the time of ovulation

Many characteristics of cervical mucus may influence criteria Inflammatory processes as well as the action of progesterone produce a thick or viscous and cellular type of mucus while estrogen and pregnant mare serum (PMS) maintain a profuse amount of clear favorable looking mucus This is also the usual finding in patients with Stein Leventhal ovaries For further discussions of this subject the reader is referred to the articles by Bergman [23] Steinberg [82] and Urdan and Kurzon [275]

Smith O W and Smith G V 1952 Endocrinology and related phenomena of the human menstrual cycle *In* Recent Progress in Hormone Research The Proceedings of the Laurentian Hormone Conference Edited by G Pincus Vol VII New York Academic Press Inc Pp 209 53

† Henderson [114] has recently made the interesting observation that this characteristic fern pattern at mid-cycle is exhibited not only by cervical mucus but also by nasal *mucus*

(f) OTHER CHANGES IN THE REPRODUCTIVE TRACT ASSOCIATED WITH OVULATION TIME

(i) *Effect of posterior pituitary extracts on myometrium* The difference in the character of myometrial contractility throughout the menstrual cycle as well as the response of uterine muscle to posterior pituitary extract was first observed by Knaus[134] Not only is this method impractical for timing ovulation but due to lack of agreement among the various workers[115 24] its validity is in question Nonetheless Knaus original observation has served as the basis in part of the *Ogino Knaus rhythm theory*[87 270]

(ii) *Cyclic variation in histology and in rhythmic contractility of the fallopian tubes* In 1924 Snyder[265] described histologic changes in the human fallopian tube during the menstrual cycle Rhythmic fluctuations in contractions of the oviduct manifesting greatest activity at ovulation time were later noted by *Seckinger and Snyder*[2 5] *Kymographic recordings* by Rubin[213] show similar effects which he interprets as peristaltic contractions

(iii) *Variations in the pH of the vagina* Zuck and Duncan[306] reported a peak in the pH of the vagina at ovulation time *However Rakoff and his associates*[191] point out that not only is there a variation in pH at various sites in the vagina but also that no consistency in the cyclic pattern can be established This subject has been reviewed more recently by Lang[74]

(iv) *Changes in vulvar fluorescence* Margolese[149] and MacDonald and Margolese[146] have reported characteristic variations in the vulvar fluorescence at the period of ovular release Because of subjective individual faults in color perception and other obvious esthetic considerations as well as the failure to detect this sign in many patients[99] this method would seem of questionable value as a routine clinical procedure to detect ovulation

(g) MISCELLANEOUS SYSTEMIC AND OTHER FLUCTUATIONS DURING THE MENSTRUAL CYCLE

(i) *Changes in levels of formed blood constituents* *Preliminary studies carried out in our laboratory*[1] have failed to confirm the observations reported by Davis and Hulit[55] of a decrease in the number of eosinophiles at mid cycle

More recently it has been demonstrated that blood platelet counts fluctuate throughout the menstrual cycle with a peak at ovulation time[80] Although of academic interest this observation does not appear to have clinical application for the use of this method would require repeated skin punctures

(ii) *Cyclic variations of other substances in the blood and urine* Edwards *et al*[76] confirmed a cyclical variation in urinary citric acid during the normal menstrual cycle previously reported by Shorr and his associates[239] but could find no consistent correlation between these changes and *estrone estradiol levels*

The concentration of serum electrolytes has been shown to fluctuate during the menstrual cycle Na K and Ca reaching a peak at ovulation time[181]

Serum vitamin A levels during the menstrual cycle *have been studied by Laurence and Sobel*[143] Maximal concentrations were observed on about day 14 and again on about day 25

Pillay[18] in India has noted an increased utilization of ascorbic acid (vitamin C) at ovulation time *Therefore if ascorbic acid is administered to saturation its excretion in the urine is decreased* when the ovum is liberated This would require that the patient be given 200 mg of ascorbic acid daily and her urine titrated Although this is a simple procedure urine collection may be cumbersome More*over the results might be misleading since aspirin* or similar substances as well as an increased fluid intake can influence this test

(iii) *Fluctuations in CO alveolar tension* Another finding of purely theoretical interest is the cyclic *variation in CO alveolar tension this shows a rise* at ovulation time[99]

(iv) *The so called electrical sign of ovulation* In 1935 Burr Hill and Allen[34] reported a striking increase in the difference of electric potential between a vaginal and suprapubic (surface) electrode at the time of ovulation in the rabbit Within the next few years similar observations were noted in other animals (rat hen sow) (cf reference 254) Moreover the confirmation of this finding in women by Burr *et al*[35-6] at Yale and later by Rock *et al*[70 01] at Harvard seemed to indicate that this was indeed a universal phenomenon which might constitute *the* sign of ovulation time so long and urgently sought for in connection with problems of human reproduction However in 1940 on the basis of a large series of carefully controlled *experiments* on human skin potentials performed in Rock s laboratory Snodgrass[53 4] proved that the electrical changes ascribed to ovulation are only *indirect effects in reality* referable to vascular and temperature changes leading to pH fluctuations which in turn affect the electric potential In a final summation of the results and interpretations of these studies Snodgrass Rock and Menkin[2 4] concluded

Bioelectric phenomena previously reported as related to ovulation are due primarily to cutaneous vascular conditions and possibly only remotely and

Vascular fluctuations during the menstrual cycle have been reported by Edwards and Duntley[75] in the skin and by Landesman *et al*[1 1] in the bulbar conjunctiva

to a slight degree to ovarian influence Local changes *in blood flow affect the focal temperature* which in turn reacts on the focal pH the fluctuations of which directly determine potential variations Because the hormonal factor is only one of several affecting vascular phenomena it is our belief that potential changes recorded thus far cannot be attributed solely to ovarian activity and that therefore the electric methods hitherto proposed are unsuitable for the detection of ovulation

In 1948 Newman[167] confirmed the findings of Snodgrass and his co workers in this connection *

(h) CLINICAL SIGNS AND SYMPTOMS AS AIDS IN EVALUATING OVULATION TIME

(i) *Mid cycle pain (Mittelschmerz)* Pain at mid cycle is experienced occasionally by some women with fair regularity by others and infrequently or not at all by most[11 12 95 156 66 137] The cause of the pain is unknown Distention of the ovarian cortex or a break in it as well as irritation of the peritoneum by follicular contents or blood have been implicated The variation in degree of intensity and in duration of the pain is wide hence the reliability of this symptom as an indication of ovulation or of the moment at which it occurs is often questionable

(ii) *Mid cycle bleeding or staining* Some women note a little vaginal bleeding or only a reddish staining associated with the theoretical time of ovulation[45 103 294] The fact that not all women bleed at mid cycle as well as the finding in some cases of occult blood reduces the value of this observation as a test for ovulation time Moreover this bleeding is often so scant that it may be detected only microscopically[175] To make the method even more sensitive workers in Israel[27] have resorted to the use of the Benzidine test However the practicability of this procedure is doubtful

(iii) *Manual palpation* Hartman[1 0 11] has timed and located the site of ovulation in the monkey by manual palpation as has Dickinson[61 5] in women However to accomplish this so many repeated examinations as well as such a high degree of tactile sensitivity and dexterity are required that few clinicians will be misled into believing that this is a useful method to apply to women

(iv) *Other miscellaneous clinical signs* Many women note the presence or an increase in mucoid vaginal discharge at times consistent with ovulation[121 5] As an indicator however it is of little value The same may be said of increased libido[62 112 271] These symptoms and others have been studied in several series of women as well as in individual cases[277 12 29 112 15 124 5 305] In regard to the observations made in the course of some of these investigations that certain subjective signs such as irritability mental depression etc appeared to show an upward trend at mid cycle it is of interest to recall the correlation between ovarian activity and psychodynamic processes reported by Benedek and Rubenstein[21 2] Kroger and Freed[136] in their book on psychosomatic gynecology summarize their views which we still consider as somewhat mystical as follows

The cyclic elaboration of ovarian hormones responsible for the menstrual cycle induces appreciable emotional response On the other hand emotional response may affect hormone elaboration Direct evidence of the effect of the separate ovarian hormones on the psyche is still inadequate Indirect evidence based on clinical observations and especially the interpretation of the unconscious material of women undergoing psychoanalysis is most significant but not conclusive There is still to be explained the relationship between the psychologic manifestations and the numerous somatic changes produced by the ovarian hormones

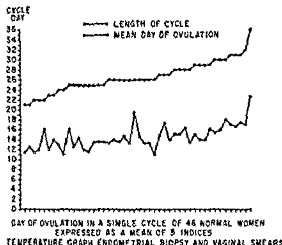
(v) *Characteristics of menstrual periods* By far the simplest clinical sign which would indicate cyclic ovulation is the regular recurrence of menstruation which is similar in quality quantity and duration of flow When this is the case one can be relatively sure that the patient is habitually ovulating and that ovulation probably takes place about two weeks prior to the onset of catamenia[196]

3 Critique

Thus we see there are many physiologic effects attributable to peak estrogen or appreciable progesterone production which are rather narrowly associated in time with ovulation Unfortunately since estrogen increases and decreases gradually and progesterone has an upward gradient which is slow until ovulation is over these several signs cannot be day specific for follicular rupture Each denotes the ovulation phase within which *at some moment* the ovum is released This phase differs in duration as well as in cycle position Similarly the signs vary in time of appearance and in duration both before and after the moment of rupture which so far as we know *gives no immediately recognizable signal*

Perhaps the most troublesome aspect of the available methods of detecting ovulation is that they are all recognizable most clearly only *after* the event For a time it was thought that one might detect the approach of the significant shift in vaginal desqua

In 1958 Parsons *et al* (*Am J Obst & Gynec* 75 121-31) reopened the question of potential changes by reporting significant differences at midcycle in premenopausal women Recordings were considered to be influenced but not materially affected by temperature and pH



DAY OF OVULATION IN A SINGLE CYCLE OF 46 NORMAL WOMEN EXPRESSED AS A MEAN OF 3 INDICES TEMPERATURE GRAPH ENDOMETRIAL BIOPSY AND VAGINAL SMEARS

Fig 5

mate as also the drop in waking temperature that sometimes precedes the rise and thus might foretell the rupture Further study however does not warrant this hopefulness It is true that Farris believes his method affords him this advantage

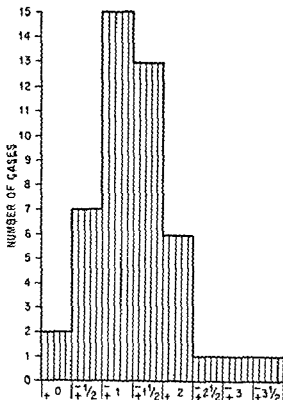
By far the most widely used clinical methods of detecting and timing ovulation are the daily basal body temperature the endometrial biopsy and the daily vaginal smear These methods when used together can be employed in clinical practice with assurance of moderate accuracy in ascertaining the ovulatory period In our study of 46 normal patients in whom the basal body temperatures endometrial biopsies and vaginal smears were utilized during the same cycle to determine the day of ovulation the mean ovulation day preceded the onset of menstruation by a fairly constant interval of 12 4 ± 1 9 days (Fig 5) In comparing the specification of the ovulation day by these three methods the span of variation shown in Figure 6 indicates that in the majority of the patients these three methods agreed within ±2 days In general the temperature graph tends to indicate an earlier ovulation day than do the vaginal smear and endometrial biopsy the latter two give about the same later date

III CONTROL OF OVULATION

Having acquired some knowledge of human oogenesis ovular maturation with follicular growth and follicular rupture the biologist would naturally inquire into the substances forces circumstances and their modes of action which favor or disturb ovarian function The gynecologist concerns himself with indices of the occurrence of ovulation or of its failure and with means by which he may induce or suppress ovulation as required for a patient s welfare From the biologist he must learn of the tools he needs and of their general capabilities He seeks these from the chemist and then attempts with them to alter the patient s physiology

Let us review what we currently imagine is Nature s way of maturing and releasing a human ovum when for the permanence of the species this may occur without endangering the individual female and by what means is the process interrupted when on the other hand the woman s safety seems to require this If we could know all those endogenous procedures the better might we imitate them for the benefit of our patients and our species

Dominating the events and their sequences are the ovarian and the pituitary hormones Stimulated perhaps by the primordial ovum its surrounding nurse cells as they proliferate into granulosa cause the adjacent stroma cells to form the surrounding thecae the internal which spontaneously secretes estrogen and then the external which strengthens and supports the follicular organ within the stroma In the blood estrogen slowly increases in concentration as during early infancy more and more follicles reach further stages of development before



MEAN VARIATION IN DAY OF OVULATION IN A SINGLE CYCLE OF 46 NORMAL WOMEN EXPRESSED AS MEAN OF 3 INDICES TEMPERATURE GRAPH ENDOMETRIAL BIOPSY AND VAGINAL SMEARS

Fig 6



FIG 7 STIGMA AFTER RUPTURE OF THE FOLLICLE (S28 706) At mid right is the granulosa and at lower left the cortical boundary of the rupture point Between these extending upward is the conglomerate of serum blood cells and evulsed granulosal cells (A F Case No 2 [91] Age 24 cycle length 28 days L M P September 28 operation October 11 day 14 of cycle) (× 150)

regressing probably from loss of their intrinsic growth potential Gradually in the little girl augmentation of blood estrogens is accompanied by the appearance in the urine of hypophyseal gonadotropin consisting apparently of only the follicle stimulating fraction (FSH) This is said to be produced in the peripherally located basophilic cells of the anterior pituitary gland [190] probably in response to either estrogen or an intermediate product which estrogen may evoke from hypothalamic cells or nerve endings and which thus via the hypophyseal stalk or the hypothalamic pituitary portal system exerts its effect within the gland itself

FSH accelerates and increases growth of ovarian follicles which of themselves have matured sufficiently to be sensitive to it

In 1931 Fevold Hisaw and Leonard [88] showed that hypophyseal gonadotropic extracts could also contain in addition to FSH a luteinizing hormone called LH This appears with puberty when estrogen from many and more mature follicles evokes the production of even larger amounts of estrogen As is the case with FSH so the amount of LH elaborated by the hypophysis is directly or indirectly affected by estrogen In synergism with FSH LH evokes within the enlarged cells of the thecae internae of ripe follicles a yellowish lipid The tissue is then said to be *luteinized*

Incipient luteinization of the theca interna may be seen in several large follicles as ovulation approaches but is most marked during the two or three days before rupture in the one bulging follicle which is doubtless the one destined to release its ovum As the estrogen content of urine is ever slowly increasing after menstruation traces of pregnanediol still thought to be the excreted metabolite of progesterone can be detected even ten days before the peak of estrogen excretion marks the onset of the still mysterious yet species essential ovulatory process [2] *

As seen in Figure 7 it is associated with not only the extrusion of the cumulus and its vital cargo but also with evulsion and discharge of sometimes more sometimes less of the granulosal layer Promptly the temporarily lowered estrogen output as reflected in urinary assays rises again and with it the production of progestin This results from the rather abrupt increase in size and activity of the now called *theca lutein* cells Within them their amplified lipids probably already elaborated the same substance that White and his associates [96] have identified in the corpus luteum of early pregnancy as steroid crystals These he and his colleagues called K *bodies* In the corpora lutea associated with early human embryos White *et al* traced the K bodies as they seemed to pass from the theca interna into the reconstructed well organized as well as well vascularized and well luteinized granulosal layer It is postulated that the K steroid is made by the granulosal cells into the progesterone which the blood stream absorbs It eems more likely that the corpus luteum of the menstrual cycle functions quite as does the yellow body of the first two weeks at least of the pregnant cycle although the former wanes during the second week and the latter during that time waxes by virtue of the new stimulant from the cytotrophoblast of the conceptus the chorionic gonadotropic hormone or HCG

We may conceive of three processes working one after the other in circular fashion to affect the ovulatory cycle (a) two pituitary gonadotropins synergistically enable the elected follicle to mature to rupture and with help from LtH then to form the corpus luteum (b) the two hormones estrogen and progesterone which the gonadotropins separately but with help each from the other evoke from the maturing follicle and in larger amounts from the corpus luteum again in synergism affect their target

* The bare possibility that this pregnanediol reflects adrenocortical secretion should not be dismissed

organs the genital epithelium and probably certain hypothalamic components and (c) neurohumoral agents from the hypothalamus influence the pituitary cells temporarily to lower production of the luteinizing hormone and increase the amount of FSH in order to complete the cycle by inducing a new crop of follicles to produce more estrogen and then at least one of them also to secrete progesterone

A Induction of Ovulation

1 Obscurity of Results

To force the recalcitrant follicles of the human ovary to rupture has been one of the most difficult problems of the clinician who would relieve amenorrhea anovulomenorrhea oligomenorrhea and the infertility associated with these results of deficient ovulation Spontaneous ovum release both isolated and recurrent is however so common in these conditions that the clinical experimenter must be overcautious in attributing even permanent cures to his therapeutic efforts

Much time and money have been spent on urinary and blood assays in attempts to locate where in the endocrine system—the hypophysis the gonad the thyroid or the adrenal—may be the primary defect which throws the whole concatenation out of kilter Except when the alert clinician detects at least suspicious signs of endocrine disorder by consideration of the external genitals and of the amount and distribution over the body of hair and fat little of more than academic interest essential to research is as yet to be gained by expensive laboratory investigations

Unfortunately the enzyme systems which doubtless mediate the action of each hormone on its target tissue be this an endocrine gland or not are almost completely obscure This seems to be why blood or urinary hormone levels may be within normal limits yet the stimulus response mechanism outlined above may fail There would seem to be variations among individuals in the elimination of hormone or in the threshold of response in the ovary for instance to FSH and in the hypothalamic-pituitary complex to estrogen For example FSH excretion may be of normal amounts and yet the circulating estrogen may bring no enlargement of follicles the follicles however will promptly grow to abnormal size if more FSH is introduced intramuscularly or into a vein

2 Known Interferences and Respective Therapy

(a) GENERAL NUTRITIVE FACTORS

Starvation whether willful as in anorexia nervosa or involuntary as in prison camps notoriously results in failure of ovulation Thus does Nature sometimes protect the individual at the expense of the species Obesity and underweight [188 28] as well as anemia also play a role in infertility

(b) HYPOPLASTIC DEFECTS

Infrequently primary amenorrhea is found attributable not to *ovarian agenesis* as in Turner's syndrome characterized by various aspects of obvious infantilism but rather to one step above the ovarian deficiency of Turner's syndrome i e to only marked hypoplasia The ovaries in these women are present and produce enough estrogen to bring them through acceptable adolescence to mental and physical maturity although sometimes—not always—with little or no libido Such ovaries may appear merely as lenticular bodies which in the adult are of the order of 3 cm long and usually not more than 1 cm in transverse diameter They may lie on the peritoneum of the infundibulo pelvic ligaments where their embryological descent was arrested

Less hypoplastic gonads with similar absence of ovulation may be in normal position suspended on their proper ligaments in normal relation to a hypoplastic uterus These ovaries are more ovoid in shape and may be not larger than about 2 × 1 cm The

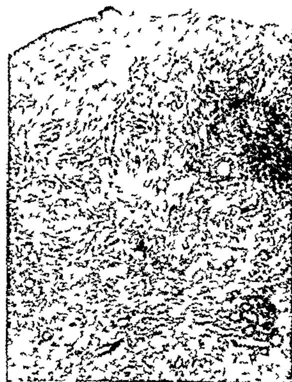


FIG 8 HYPOPLASTIC OVARY (S54-4665) Extreme cortical fibrosis One graafian follicle and in this section a normal number of primordial follicles (× 75)

number of primitive follicles may seem to be less than or within normal limits but apparently none of them responds sufficiently to FSH which is at least excreted in such amounts as to produce sufficient estrogen to evoke LH and then to progress to maturation Invariably there is cortical fibrosis (Fig 8)

Again unlike the situation in Turner s syndrome where bodily divergencies reflect the ovarian deficiency the diagnosis of such marked hypoplasia is made only on inspection by laparotomy or culdoscopy [131] In our hands these individuals may be made to menstruate with cyclic estrogen and progesterone therapy but like others we [163] have not been able to stimulate follicular growth

As patients with primary amenorrhea may have abnormally high FSH urinary titers (Turner s syndrome) so also do some unfortunate women with secondary amenorrhea in their late twenties or thirties In them the menopause has occurred As with ovarian agenesis the condition must be considered irreversible as far as ovulation is concerned We can only wonder what causes premature aging of ova or their destruction

(c) POLYCYSTIC DISEASE

A much more favorable ovarian fault causing long *periods of secondary amenorrhea is that which was* very helpfully defined by Stein and Leventhal [261] in 1935 and more recently reviewed by Stein [59] Stein Leventhal ovaries are often palpable being larger than the ordinary ovary for they invariably contain several full sized follicles and many others with diameters of 3 5 mm In some instances signs of masculinization are detectable and 17 ketosteroid excretion may be increased These polycystic ovaries when not palpable as such may be delineated by pneumoroentgenography [258] as also by culdoscopy [131] Resection of them will bring fairly prompt recurrent ovulation in about 30 per cent of cases and complete recovery of normal function in perhaps one third of these However it should be pointed out that many of these patients later revert to their previous anovulatory status [204]

Since the cause of anovulation in this condition is unknown one cannot explain either successes or failures One may theorize that functional imbalance in one or more persisting follicles interferes with the normal call by estrogen *for the LH* which *evokes the* indispensable maturing luteinization of the theca interna and *that resection* of the ovaries in the successful cases removes the inhibiting follicles which are *indistinguishable from* normal ones and misses them in the others One may also contemplate the possibility that those hilus cells which probably produce at least some of the female androgens and which occasionally give rise to androgenic tumors well described by Sternberg [63] may produce too much androgen whether or not the 17 ketosteroids are high or possibly peculiar androgens in amounts that block the reaction of pituitary LH and that in the cases of cure the resection of the follicles encroaches on the hilus to such a degree as to remove them It would seem because of the failure of thecal luteinization and also because of the surface bulging of so many follicles through the more fibrotic cortex present in the Stein Leventhal ovary that mere incision of it with removal of a part was not the significant factor This is substantiated by the occurrence of ovulation in monkeys in spite of the artificial induction of cortical fibrosis [2 4]

These polycystic ovaries are the ones that also respond in about 20 per cent of cases to the so called stimulating doses of x rays so ardently championed by Kaplan [129] and his followers We are no longer among them although we acknowledge the frequent curative effect resulting possibly by virtue of radiation destruction of the same theoretically inhibitory follicles or hylar cells mentioned above [203] We fear the future species defects to come from the undoubtedly consequent cacogenic inheritable mutations in all the remaining ova [162] This of course may not be apparent until children both of whom descend from irradiated forebears mate and both hand on damaged genes Nor need all the offspring of such be abnormal to vitiate the procedure

(d) INFLAMMATION AND ENDOMETRIOSIS

Menstrual dysfunction is well known to occur in acute infections and pelvic inflammatory disease as also in cases of extensive ovarian endometriosis Cortical pathology may be considered the basis of the causal uncertainty of ovulation and/or luteal function Surgical repair of damaged ovaries can be expected to relieve these conditions

(e) HYPOPHYSEAL FAILURE

Bodily disturbances affecting the function of the anterior pituitary gland so that significant alterations are made in its secretions usually result in a failure of ovulation Conditions arising in this master gland itself may be similarly suppressive One thinks of *acidophilic adenomas* (*acromegaly*) basophilic adenomas (Cushing s disease) craniopharyngiomas chromophobic adenomas Simmonds disease (and its variation associated with obstetric shock i e postpartum pituitary necrosis or Sheehan s disease) the Chiari Frommel syndrome the Laurence Moon Biedl syndrome and panhypopituitarism The hor



Fig 9 Follicular stimulation by FSH (Gonadophysin–Searle) (S50-3008) At laparotomy—uterus in upper half both enlarged ovaries in lower half No corpus luteum found



Fig 10 Cut surface of ovary of Figure 9 stimulated with FSH (Gonadophysin–Searle) Many very large follicles

monal relationships in these specific disturbances of the pituitary gland are discussed elsewhere in this volume[256] Attention is focused here on the stimulation and/or attempted replacement of the hypophyseal secretions which are concerned with the provocation of ovulation

With FSH from pregnant mare serum (PMS) or sheep pituitary gland as also with human chorionic gonadotropin (HCG) veterinary biologists[106, 30] are easily able to provoke ovulation during anestrus in several mammals below the primates In the human however Erving and his co-workers[39] have reported discouraging results with Gonadin Cutter (also a preparation of equine gonadotropic hormone)

In 1951 findings similar to those of others were obtained in women by Mulligan and Rock[64] Using a potent FSH preparation (Gonadophysin Searle) intramuscularly for three to four days and intravenously on the last day of treatment they succeeded in obtaining only extreme development of follicles but no rupture even though in several instances many large follicles were found to have apparent thecal luteinization (Figs 9 10 11)



Fig. 11 Cross section of ovary stimulated by FSH (Gonadophysin–Searle) (S50-3008)

[illegible] [illegible] et al.[illegible] have reported more gratifying results with Premarin-Ayerst (a preparation containing conjugated estrogens from the urine of pregnant mares). Our own trials with Premarin have not been successful.

Mention has been made of the fact that through the neurohumoral influence of the hypothalamus pituitary gonadotropic activity is affected. The possible modification of this hypothalamic action by stimuli to the hypothalamus from the central nervous system has been discussed with favor by Donovan and Harris,[8] but is still adversely considered by Zuckerman.[30] In women disruption of regular ovulation giving even long periods of failure is not uncommonly so closely related in time to wide change in place of abode as to warrant the assumption that environmental factors are in play. Sometimes the difference in meteorologic milieu is so extreme as to suggest that through the autonomic nervous system the humoral function of the hypothalamus is disturbed. If such be the case adaptation to the new surroundings with time may be expected. In other instances, as are frequent in girls after entering college or hospital training school, it would seem most likely that cortical influences are involved. One postulates a similar mechanism when anovulation follows promptly on what a woman thinks is for her a tragic event. The neurosis of anorexia nervosa may cause its associated amenorrhea via a similar cortical effect, but in this condition we also have to consider the possible role of avitaminosis or malnutrition. In all cases of obscure secondary amenorrhea a careful history is required. When emotional factors give suspicion of involvement,[211, 20] the therapeutic possibilities of psychotherapy should be restrainedly and gently explored by the gynecologist and appropriate action slowly and tactfully taken.

In 1953 Netter and Lambert[165] of Paris reported a brave and interesting attack on ovulatory failure originally described by them in 1943, which has not and probably will not achieve much general use in this country. By means of procaine rather skillfully injected directly into the two superior cervical sympathetic ganglia lying on the anterior surface of the [illegible] [illegible] ovulation was induced and generally [illegible] regular in [illegible] three-fifths of 111 patients [illegible]. These excellent results are attributed [illegible] of the sympathetic innervation of the [illegible] which effect permits parasympathetic [illegible] activity tow[illegible] [illegible] [illegible] It [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

(f) OTHER ENDOCRINE FAILURE: THE ADRENAL CORTEX AND THE THYROID

When dysfunction of the adrenal cortex results in disruption of the pituitary-ovarian axis, signs of electrolyte imbalance and, more obviously, of masculinization are easily detectable.

The excretion of even moderately excessive amounts of 17-ketosteroids, as is occasionally found with ovulatory failure even though the ovaries be not enlarged, may suggest such an increased blood content as directly or indirectly to suppress the production of that amount of LH which will induce the follicular thecal layer to accomplish complete maturation. Cortisone or perhaps hydrocortisone by mouth in doses of from 25 to 100 mg per day may depress adrenocortical production of 17-ketosteroids and thus possibly permit estrogen to evoke LH. Such is the explanatory theory in those few cases in which ovulation may follow the administration of cortisone. Adrenocortical tumors must always be suspected and appropriately sought after and excised when androgenicity accompanies signs of ovulatory failure. Attempts have been made to detect, by fractional steroid analysis of urine from patients thought to have Stein-Leventhal syndrome, whether the suspected interference is of adrenal or possibly of hylar cell origin.

Deleterious effects of thyroid disorder may be somewhat less overt than those from hyperactivity in the adrenals. As for patients in general, so particularly in all cases of incomplete follicular maturation, thyroid function must be carefully appraised by one means or another. The appropriate method is dependent on the clinician's examining acumen, on the availability and reliability of laboratory procedures, and on the financial resources of the patient. Determinations of basal metabolic rate, like those of blood cholesterol, are likely to be less informative than accurate assay of blood for protein-bound iodine or measurement of iodine 131 uptake.[130, 8] Since the thyroid hormone, through one or more of its metabolites, is a growth factor, rectification of either increased or decreased thyroid function is called for. It appears that in some instances added thyroid may also be helpful if autogenous values are only barely above the lower limit of normal.[4] Therapeutic results should be carefully assessed by repeat tests.

B. Suppression of Ovulation

The suppressive effect of estrogen, of progesterone, or of testosterone is well known,[11] but these hormones either fail to prevent irregular anovulatory flow, or require such comparatively large amounts [illegible] [illegible]ncy as to occasion side effects unac[illegible]

ceptable to many women Radiation will stop ovulation but by precipitating the menopause Reserpine a Rauwolfian derivative has been found to block ovulation in monkeys [59] as has the tranquilizing chlorpromazine in a small percentage of women [97] but the pharmacologic effects of these substances make them undesirable The subject of suppression of ovulation has been discussed lately by Zuckerman [310] and by Millman and Hartman [157]

Recent and current experimentation in animals and women gives promise that a useful ovulation inhibitor may be found among one or more of several synthetic steroids which manifest extremely potent progestational qualities and if properly used will be without objectionable effects on menstruation or subsequent ovarian function [185 186 0) 197 96] Karl Sax has recently directed attention to the overpopulated areas of the world and the need for a fertility indicator [2 0]

BIBLIOGRAPHY

1 Abarbanel A R 1946 Artificial reproduction of the cyclic changes in cervical mucus in human castrates In Trans Am Soc for the Study of Sterility Portland Oregon Edited and published by The Western Journal of Surgery Publishing Company Pp 46-62
2 Albright F Butler A M Hampton A O and Smith P 1937 Syndrome characterized by osteitis fibrosa disseminata areas of pigmentation and endocrine dysfunction with precocious puberty in females Report of five cases New Eng J Med 216 727-40
3 Albright F Scoville B and Sulkowitch H W 1938 Syndrome characterized by osteitis fibrosa disseminata areas of pigmentation and a gonadal dysfunction Further observations including the report of two more cases Endocrinology 22 411 21
4 Allan F D 1958 The embryology of the reproductive system Chapter III in The Endocrinology of Reproduction Edited by Joseph T Velardo New York Oxford University Press 21 42
5 Allen E 1922 The oestrous cycle in the mouse Am J Anat 30 297 371
6 ——— 1923 Ovogenesis during sexual maturity Am J Anat 31 439 81
7 Allen E and Creadick R N 1937 Ovogenesis during sexual maturity The first stage mitosis in the germinal epithelium as shown by the colchicine technique Anat Rec 69 191 5
8 Allen E Pratt J P Newell Q U and Bland L J 1930 Human ova from large follicles including a search for maturation divisions and observations on atresia Am J Anat 46 1 53
9 ——— 1930 Human tubal ova related early corpora lutea and uterine tubes Carn Inst Wash Pub 414 Contrib Embryol 22 No 127 45 76
10 Anderson A E 1956 Pregnancy at age of fifty six years Report of a case Obst & Gynec 7 494 5
11 Anderson C W 1933 Natural avoidance of conception Col Med 30 223 7
12 ——— 1934 Teaching the patient to observe the symptoms of ovulation Col Med 31 344 8
13 Astwood E B 1941 The regulation of corpus luteum function by hypophysial luteotrophin Endocrinology 28 309 20
14 Astwood E B and Fevold H L 1939 Action of progesterone on the gonadotropic activity of the pituitary Am J Physiol 127 192 8
15 Barjaktarovitch S S 1954 Le mecanisme de l ovulation chez la femme (Mechanism of ovulation in the human female) English abstract in Congress Number Internat Congress of Gynaec and Obst Geneva July 26 31 1954 Excerpta Medica (Section X) 7 339
16 Barton D S 1940 A study of temperature and electric potentials in the menstrual cycle Yale J Biol & Med 12 503 23
17 Barton M and Wiesner B P 1945 Waking temperature in relation to female fecundity Lancet 2 663 8
18 ——— 1945 Thermogenic effect of progesterone Lancet 2 671 2
19 Belonoschkin B 1954 Spermiogenesis in elderly men Fert & Ster 5 182 92
20 Benedek T 1952 Infertility as a psychosomatic defense Fert & Ster 3 527 41
21 Benedek T and Rubenstein B B 1939 The correlations between ovarian activity and psychodynamic processes I The ovulative phase Psychosom Med 1 245 70
22 ——— 1939 The correlations between ovarian activity and psychodynamic processes II The menstrual phase Psychosom Med 1 461 85
23 Bergman P 1950 Sexual cycle time of ovulation and time of optimal fertility in women Studies on basal body temperature endometrium and cervical mucus Acta Obst et Gynec Scandinav 29(Suppl 4) 5 139
24 Blandau R J 1955 Ovulation in the living albino rat Fert & Ster 6 391-404
25 Braunsberg H Stern M I and Ferrarotti N G 1956 Estudio hormonal del ciclo menstrual normal por nuevos metodos Rev Argent de Endocrin y Metab 2 13 18
26 Brewer J I and Jones H O 1947 The time of ovulation Am J Obst & Gynec 53 637-44
27 Bromberg Y M and Bercovici B 1956 Occult intermenstrual bleeding about the time of ovulation Fert & Ster 7 71 9
28 Brown W 1955 Current Reviews The ovary Fert & Ster 6 476 89
29 Bryant R D 1939 Exploded theories of ovulation The various notions on when ovulation occurs and their bases Ohio State Med J 35 955 8
30 Bullough W S 1942 Oogenesis and its relation to the oestrous cycle in the adult mouse J Endocrin 3 141 9
31 ——— 1942 The method of growth of the follicle and corpus luteum in the mouse ovary J Endocrin 3 150 56
32 ——— 1943 The effects of oestrone on the ovary of the mouse J Endocrin 3 235 43
33 ——— 1946 Mitotic activity in the adult female mouse Mus musculus L A study of its relation to the oestrous cycle in normal and abnormal conditions Phil Trans Roy Soc London Series B 231 453 516
34 Burr H S Hill R T and Allen E 1935 Detection of ovulation in the intact rabbit Proc Soc Exper Biol & Med 33 109 11
35 Burr H S Musselman L K Barton D S and Kelly N B 1937 A bio electric record of human ovulation Science 86 312

Recently Kupperman *et al*[139] have reported more gratifying results with Premarin Ayerst (a preparation containing *conjugated estrogens from the urine* of pregnant mares). Our own trials with Premarin have not been successful.

Mention has been made of the fact that through the neurohumoral influence of the hypothalamus pituitary gonadotropic activity is affected. The possible modification of this hypothalamic action by stimuli to the hypothalamus from the central nervous system has been discussed with favor by Donovan and Harris[67] but is still adversely considered by Zuckerman.[309] In women disruption of regular ovulation giving even long periods of failure is not uncommonly so closely related in time to wide change in place of abode *as to warrant the assumption that* environmental factors are in play. Sometimes the difference in meteorologic milieu is so extreme as to suggest that through the autonomic nervous system the humoral function of the hypothalamus is disturbed. If such be the case adaptation to the new surroundings with time may be expected. In other instances as are frequent in girls after entering college or hospital training school it would seem most likely that cortical influences are involved. One postulates a similar mechanism when anovulation follows promptly on what a woman thinks is for her a tragic event. The neurosis of anorexia nervosa may cause its associated amenorrhea via a similar cortical effect but in this condition we also have to consider the possible role of avitaminosis or malnutrition. In all cases of obscure secondary amenorrhea a careful history is required. When emotional factors give suspicion of involvement[211, 20] the therapeutic possibilities of psychotherapy should be restrainedly and gently explored by the gynecologist and appropriate action slowly and tactfully taken.

In 1953 Netter and Lambert[165] of Paris reported a brave and interesting attack on ovulatory failure originally described by them in 1943 which has not and probably will not achieve much general use in this country. By means of procaine rather skillfully injected directly into the two superior cervical sympathetic ganglia lying on the anterior surface of the cervical vertebrae ovulation was induced and generally became regular in *c* three fifths of 111 patients treated. These excellent results are attributed to paralysis of the sympathetic innervation of the hypothalamus which effect permits parasympathetic nerves to rectify hypothalamic activity toward improved hypophyseal function. It is reported by Greenhill[165] that accurate placement is evidenced by myosis enophthalmos lacrimation and congestion of the face on the affected side. There may be also transient paralysis of the palate and respiratory difficulty which last about an hour.

(f) OTHER ENDOCRINE FAILURE THE ADRENAL CORTEX AND THE THYROID

When dysfunction of the adrenal cortex results in disruption of the pituitary ovarian axis signs of electrolyte imbalance and more obviously of masculinization are easily detectable.

The excretion of even moderately excessive amounts of 17 ketosteroids as is occasionally found with ovulatory failure even though the ovaries be not enlarged may suggest such an increased blood content as directly or indirectly to suppress the production of that amount of LH which will induce the follicular thecal layer to accomplish complete maturation. Cortisone or perhaps hydrocortisone by mouth in doses of from 25 to 100 mg per day may depress adrenocortical production of 17 ketosteroids and thus possibly permit estrogen to evoke LH. Such is the explanatory theory in those few cases in which ovulation may follow the administration of cortisone. Adrenocortical tumors must always be suspected and appropriately sought after and excised *when androgenicity accompanies signs of ovulatory* failure. Attempts have been made to detect by fractional steroid analysis of urine from patients thought to have Stein Leventhal syndrome whether the suspected interference is of adrenal or possibly of hylar cell origin.

Deleterious effects of thyroid disorder may be somewhat less overt than those from hyperactivity in the adrenals. As for patients in general so particularly in all cases of incomplete follicular maturation thyroid function must be carefully appraised by one means or another. The appropriate method is dependent on the clinician's examining acumen on the availability and reliability of laboratory procedures and on the financial resources of the patient. Determinations of basal metabolic rate like those of blood cholesterol are likely to be less informative than accurate assay of blood for protein bound iodine or measurement of iodine 131 uptake.[150] Since the thyroid hormone through one or more *of its metabolites is a* growth factor rectification of either increased or decreased thyroid function is called for. It appears that in some instances added thyroid may also be helpful if autogenous values are only barely above the lower limit of normal.[46] Therapeutic results should be carefully assessed by repeat tests.

B Suppression of Ovulation

The suppressive effect of estrogen of progesterone or of testosterone is well known[10] but these hormones either fail to prevent irregular anovulatory flow or require such comparatively large amounts for oral efficiency as to occasion *side effects* unac

menstrual cycle and ovariectomy Am J Obst & Gynec 57 501 9
76 Edwards M S Gray C H and Wood M E 1950 Citric acid and the excretion of oestrone and oestradiol J Endocrin 6 440 48
77 Erving H W Sears C and Rock J 1940 Clinical experiences with equine gonadotropic hormone Am J Obst & Gynec 40 695-713
78 Escomel E 1939 Lovaire de Lima Medina (la plus jeune mere du monde) Presse Med 47 1648
79 Evans H M and Swezy O 1931 Ovogenesis and the normal follicular cycle in adult mammalia Mem Univ Calif 9 119 224
80 Everett J W Sawyer C H and Markee J E 1949 A neurogenic timing factor in control of the ovulatory discharge of luteinizing hormone in the cyclic rat Endocrinology 44 234 50
81 Everett N B 1943 Observational and experimental evidences relating to the origin and differentiation of the definitive germ cells in mice J Exper Zool 92 49 91
82 —— 1945 The present status of the germ cell problem in vertebrates Biol Rev 20 45-55
83 Farris E J 1946 A test for determining the time of ovulation and conception in women Am J Obst & Gynec 52 14 27
84 —— 1950 Human Fertility and Problems of the Male White Plains New York The Author s Press Inc
85 Fevold H L 1944 The gonadotrophic function of the pituitary gland *In* The Chemistry and Physiology of Hormones Edited by F R Moulton Washington D C Publication of the American Association for the Advancement of Science Pp 152 61
86 Fevold H L Hisaw F L and Leonard S L 1931 The gonad stimulating and the luteinizing hormones of the anterior lobe of the hypophysis Am J Physiol 97 291 301
87 Fleck S Snedeker E F and Rock J 1940 The contraceptive safe period A clinical study New Eng J Med 223 1005-9
88 Fluhmann C F 1934 A new procedure for the demonstration of estrin in the blood of women Endocrinology 18 705 13
89 —— 1956 Age at inception of menstruation Chapter 6 in The Management of Menstrual Disorders Philadelphia London W B Saunders Co Pp 61-8
90 —— 1956 The Management of Menstrual Disorders Philadelphia London W B Saunders Co Pp 70 73
91 —— 1956 The Management of Menstrual Disorders Philadelphia London W B Saunders Co Pp 135 6
92 —— 1956 The Management of Menstrual Disorders Philadelphia London W B Saunders Co P 137
93 Forbes T R 1950 Systemic plasma progesterone level during the human menstrual cycle Am J Obst and Gynec 60 180 86
94 Fuss A 1911 Ueber extraregionare Geschlechtszellen bei einem men chlichen Embryo von 4 Wochen Anat Anz 39 407 9
95 —— 1912 Uber die Geschlechtszellen des Menschen und der S ugetiere Arch f mikr Anat 81 1 23
96 Garcia C R Pincus G and Rock J 1958 Effects of three 19 nor steroids on human ovulation and menstruation Am J Obst & Gynec 75 82 97
97 Goldhar A Grody M H and Masters W H 1952 The vaginal smear as an ovulatory index Fert & Ster 3 376 93
98 Goodland R L and Pommerenke W T 1952 Cyclic fluctuations of the alveolar carbon dioxide tension during the normal menstrual cycle Fert & Ster 3 394 401
99 Greenblatt R B 1951 The clinical application of vulvar fluorescence Fert & Ster 2 467 73
100 Greulich W W Morris E S and Black M E 1943 The age of corpora lutea and timing of ovulation *In* Proceedings of the Conference on Problems of Human Fertility Sponsored by the National Committee on Maternal Health January 15-16 1943 New York City Edited by Earl T Engle Menasha Wisconsin George Banta Publishing Co Pp 87 66
101 Gustavson R G Mason L W Hays E E Wood T R and D Amour F E 1938 The quantitative determination of estrogenic substances in normal female urine during the menstrual cycle Am J Obst & Gynec 35 115-23
102 Haggstrom P 1921 Zahlenmassige Analyse der Ovarien eines 22 jahrigen gesunden Weibes Upsala Lakareforenings Forhandlingar 26 5 and 6 W 52 pp
103 Hain A M 1934 Ovulation and the human sex cycle Brit Med J 1 454 5
104 Halbrecht I 1945 Ovarian function and body temperature Lancet 2 668 9
105 Hamilton W J 1944 Phases of maturation and fertilization in human ova J Anat 78 1-4
106 Hammond J 1950 Problems concerning the transplantation of fertilized ova or artificial pregnancy An de la Fac de Med Montevideo 35 810 19
107 —— 1955 Hormones in relation to fertility in farm animals Brit Med Bull 11 165 8
108 Harris G W and Jacobsohn D 1952 Functional grafts of the anterior pituitary gland Proc Roy Soc of London Series B 139 263 76
109 Hartman C G 1932 Ovulation and the transport and viability of ova and sperm in the female genital tract Chapter XIV *in* Sex and Internal Secretions A Survey of Recent Research Edited by Edgar Allen Baltimore William & Wilkins Co Pp 647 58
110 —— 1932 Studies in the reproduction of the monkey Macacus (Pithecus) rhesus with special reference to menstruation and pregnancy Carn In t Wash Pub 433 Contrib Embryol 23 No 134 1 161
111 —— 1933 Pelvic (rectal) palpation of the female monkey with special reference to the ascertainment of ovulation time Am J Obst & Gynec 26 600 608
112 —— 1936 Time of Ovulation in Women A Study on the Fertile Period in the Menstrual Cycle Baltimore Williams & Wilkins Co
113 —— 1955 How variable is the functional life of the human corpus luteum menstruationis? World Ob t & Gynec 7 323-35
114 Henderson I D 1956 Cyclical changes in female nasal mucus J Clin Endocrin & Metab 16 905 9
115 Henry J S and Browne J S L 1943 The contractions of the human uterus during the menstrual cycle The effect of progesterone and posterior pituitary extract upon the motility of the human uterus Am J Obst & Gynec 45 927-49
116 Henshaw P S 1953 Physiologic control of fertility Science 117 572 82

36 Burr H S Musselman L K Barton D S and Kelly N B 1937 Bio electric correlates of human ovulation Yale J Biol & Med 10 155 60
37 Buxton C L 1951 Discussion of Evaluation of the basal body temperature An analysis of 1012 basal body temperature recordings Siegler S L and Siegler A M Fert & Ster 2 287 301 P 300
38 Buxton C L and Atkinson W B 1948 Hormonal factors involved in the regulation of basal body temperature during the menstrual cycle and pregnancy J Clin Endocrin 8 544 9
39 Buxton C L and Engle E T 1950 Time of ovulation A correlation between basal temperature the *appearance of the endometrium and the appearance of the ovary* Am J Obst & Gynec 60 539 51
40 Campos da Paz A 1953 The crystallization test as a guide to the treatment of cervical hostility Fert & Ster 4 137-48
41 Cary W H 1940 Experience with artificial im*pregnation in treating sterility Report of thirty five* cases J Am Med A 114 2183 7
42 Chang M C and Pincus G 1951 Physiology of fertilization in mammals Physiol Rev 31 1 26
43 Cleghorn R A and Graham B F 1949 Manifestations of altered autonomic and humoral function in psychoneuroses *In* Recent Progress in Hor*mone Research The Proceedings of the Laurentian* Hormone Conference Edited by G Pincus Vol IV New York Academic Press Inc Pp 323 62
44 Clift A F 1945 Observations on certain rheological properties of human cervical secretion Proc Roy Soc Med 39 1 9
45 Cohen M R Stein I F Sr and Kaye B M 1952 *Spinnbarkeit* a characteristic of cervical mucus Significance at ovulation time Fert & Ster 3 201 9
46 Comninos A C 1956 Thyroid function and therapy in reproductive disturbances Obst & Gynec 7 260 67
47 Corner G W 1923 Ovulation and menstruation *in Macacus rhesus* Carn Inst Wash Pub 332 Contrib Embryol 15 No 75 73 101
48 ——— 1938 The sites of formation of estrogenic substances in the animal body Physiol Rev 18 154 72
49 Corner G W Hartman C G and Bartelmez G W 1945 Development organization and break*down of the corpus luteum in the rhesus monkey* Carn Inst Wash Pub 557 Contrib Embryol 31 No 204 117-46
50 D Amour F E 1940 Further studies on hormone excretion during the menstrual cycle Am J Obst & Gynec 40 958 65
51 ——— 1943 Comparison of methods used in determining time of ovulation J Clin Endocrin 3 41 8
52 Davis K B 1929 Factors in the Sex Life of 2,200 Women New York Harper s
53 Davis M E 1946 The clinical use of oral basal temperatures J Am Med A 130 929 32
54 Davis M E and Fugo N W 1948 The cause of physiologic basal temperature changes in women J Clin Endocrin 8 550 63
55 Davis M E and Hulit B E 1949 Changes in circulating eosinophils in women during the menstrual cycle and reproduction J Clin Endocrin 9 714 24
56 de Allende I L C and Orias O 1950 Cytology of the Human Vagina New York Paul B Hoeber Inc
57 de Allende I L C Shorr E and Hartman C G 1945 A comparative study of the vaginal smear cycle of the rhesus monkey and the human Carn Inst Wash Pub 557 Contrib Embryol 31 No 198 1 26
58 Decker A 1951 Culdoscopic observations on the *tubo-ovarian mechanism of ovum reception* Fert & Ster 2 253-9
59 De Feo V J and Reynolds S R M 1956 Modification of the menstrual cycle in rhesus monkeys by reserpine Science 124 726 7
60 de Graaf R 1672 De Mulierum Organis Generationi Inservientibus Cited by Adelmann H B 1933 *The De Ovorum Gallinaceorum Generationis* Primo Exordio Progressuque et Pulli Gallinacei Creationis Ordine of Volcher Coiter Ann Med Hist N S 5 327-41 444 57
61 Del Castillo E B Argonz J and Munini C G 1948 Cytological cycle of the urinary sediment and its parallelism with the vaginal cycle J Clin Endocrin 8 76-87
62 Dempsey E W 1948 III Histochemical and histophysical methods in hormone research 5 The chemical cytology of endocrine gland *In* Recent Progress in Hormone Research The Proceedings of the Laurentian Hormone Conference Edited by G Pincus Vol III New York Academic Press Inc Pp 127 57
63 Dempsey E W and Bassett D L 1943 Observations on the fluorescence birefrigence and histochemistry of the rat ovary during the reproductive cycle Endocrinology 33 384-401
64 Dickinson R L 1949 Atlas of Human Sex Anatomy Second Edition Baltimore Williams & Wilkins Co
65 ——— 1937 The technic of timing human ovulation by palpable changes in ovary tube and uterus Am J Obst & Gynec 33 1027 33
66 Diddle A W 1948 Relation of intermenstrual symptoms and signs to ovulation as determined by basal body temperatures Am J Obst & Gynec 56 537-40
67 Donovan B T and Harris G W 1955 Neurohumoral mechanisms in reproduction Brit Med Bull 11 93 7
68 Doyle J B 1951 Exploratory culdotomy for observation of tubo ovarian physiology at ovulation time Fert & Ster 2 475 86
69 ——— 1954 Ovulation and the *effects of selective* uterotubal denervation Direct observations by culdotomy Fert & Ster 5 105-30
70 Dubreuil G 1946 L ovaire feminin envisage a la lueur des recentes acquisitions morphologiques et histophysiologiques *In* Les Trouble de l Ovulation I Rapports 10e Congres Français de Gynecologie (Lyon 27 29 Mai *1946) Paris L Expan*sion Scientifique Française Pp 1 25
71 Duryee W R 1954 Microdissection studies on human ovarian eggs Trans N Y Acad Sci Ser II 17 103 08
72 Eastman N J 1956 Williams Obstetrics Eleventh Edition New York Appleton Century Crofts Inc P 56
73 ——— 1956 Williams Obstetrics Eleventh Edition New York Appleton Century Crofts Inc P 66
74 ——— 1956 Williams Obstetrics Eleventh Edition New York Appleton Century Crofts Inc P 85
75 Edwards E A and Duntley S Q 1949 Cutaneous vascular changes in women in reference to the

Français de Gynécologie (Lyon 27 29 Mai 1946) Paris L'Expansion Scientifique Française Pp 26-68

161 Morse A H and van Wagenen, G 1936 Observations upon ovulation in primates Am J Obst & Gynec 32 823-32

162 Muller H J 1954 Damage to posterity caused by irradiation of the gonads Am J Obst & Gynec 67 467 83

163 Mulligan W J Horne H W Jr and Rock J 1952 Cyclic oral therapy of menstrual disorders Fert. & Ster 3 328-33

164 Mulligan W J and Rock J 1951 The effects of sheep gonadotropins on the human ovary Read before the Section on Obstet and Gynec One Hundredth Annual Session American Med Assn Atlantic City N J June 13 1951 *in* J Amer Med A 145 1160

165 Netter A and Lambert A 1953 Initiation of ovulation by infiltration of superior cervical sympathetic ganglion Cited in The Year Book of Obstetrics and Gynecology 1953 1954 Edited by J P Greenhill Chicago Ill The Year Book Publishers Pp 300 301

166 Newell J W and Rock J 1952 Upper age limit of parturition A review of the literature Am J Obst & Gynec 63 875-6

167 Newman H F 1948 Ovulation studies with direct current potentials Am J Obst & Gynec 56 901-6

168 Nieburgs H E 1945 Body temperature—a diagnostic aid in menstrual disorders and sterility J Obst & Gynaec Brit Emp 52 435 62

169 Noble R L and Plunkett E R 1955 Biology of the gonadotrophins Brit Med Bull 11 98-101

170 Noyes R W Hertig A T and Rock J 1950 Dating the endometrial biopsy Fert & Ster 1 3 25

171 Ogino K 1930 Ovulationstermin und Konzeptionstermin Zentralbl f Gynak 54 464 79

172 Palmer A 1949 The diagnostic use of the basal body temperature in gynecology and obstetrics Obst & Gynec Surv 4 1 26

173 Palmer R 1938 Discussion of La presence du glycogene dans la muqueuse uterine de femme un des tests d action de la progesterone Mocquot P and Moncard R Bull de la Soc de Gynec et d Obst de Paris 27 503 7

174 Palmer R and Devillers J 1939 Action thermique des hormones sexuelles chez la femme Comptes Rendus des Séances de la Soc de Biol 130 895 6

175 Papanicolaou G N 1933 The sexual cycle in the human female as revealed by vaginal smears Am J Anat 52 519-637

176 —— 1945 Some characteristic changes in the consistency of the uterine secretion Anat Rec 91 293

177 —— 1946 A general survey of the vaginal smear and its use in research and diagnosis Am J Obst & Gynec 51 316-28

178 Papanicolaou G N Traut H F and Marchetti A A 1948 The Epithelia of Woman s Reproductive Organs A Correlative Study of Cyclic Changes New York The Commonwealth Fund

179 Payne S and Skeels R F 1954 *Fertility as revealed* by artificial insemination Fert & Ster 5 32 9

180 Pepper H and Lindsay S 1956 Elevation of platelets in mid-cycle an indication of ovulation Science 124 180-81

181 Phillips R S McCoord A B and Pommerenke W T 1952 Serum electrolytes in the menstrual cycle Fert & Ster 3 402 18

182 Pillay A P 1946 Vitamin C test for ovulation Human Fertility 11 109 10

183 Pincus G 1936 The Eggs of Mammals New York The Macmillan Company

184 —— 1940 Superovulation in rabbits Anat. Rec 77 1 8

185 Pincus G Chang M C Hafez E S E Zarrow M X and Merrill A 1956 Effects of certain 19 nor steroids on reproductive processes in animals Science 124 890-91

186 Pincus G Chang M C Zarrow M X Hafez E S E and Merrill A 1956 Studies of the biological activity of certain 19 nor steroids in female animals Endocrinology 59 695-707

187 Pincus G and Saunders B 1939 The comparative behavior of mammalian eggs in vivo and in vitro VI The maturation of human ovarian ova Anat Rec 75 537-45

188 Pommerenke W T 1944 Criteria of ovulation West J Surg Obst & Gynec 52 416-31

189 Pommerenke W T and Viergiver E 1946 Cervical mucus and the menstrual cycle *In* The Problem of Fertility Proceedings of the Conference on Fertility Held under the Auspices of the National Committee on Maternal Health Edited by E T Engle Princeton N J Princeton University Press Pp 102-18

190 Purves H D and Griesbach W E 1954 The site of follicle stimulating and luteinising hormone production in the rat pituitary Endocrinology 55 785-93

191 Rakoff A E Feo L G and Goldstein L 1944 The biological characteristics of the normal vagina Am J Obst & Gynec 47 467 94

192 Riley G M Dontas E and Gill B 1955 Use of serial vaginal smears in detecting time of ovulation Fert & Ster 6 86-102

193 Rock J 1941 Medical Progress Ovulation New Eng J Med 225 910-17

194 —— 1943 Medical and biological aspects of contraception The scientific case against rigid legal restrictions on medical birth control advice Clinics 1 1598 1611

195 Rock J and Bartlett M K 1937 Biopsy studies of human endometrium Criteria of dating and information about amenorrhea menorrhagia and time of ovulation J Am Med A 108 2022-8

196 Rock J Bartlett, M K and Matson D D 1939 The incidence of anovulatory menstruation among patients of low fertility Am J Obst & Gynec 37 3-12

197 Rock J Garcia C R and Pincus G 1957 Synthetic progestins in the normal human menstrual cycle *In* Recent Progress in Hormone Research The Proceedings of the Laurentian Hormone Conference Edited by G Pincus Vol XIII New York, Academic Press Inc Pp 323-46

198 Rock J and Hertig A T 1942 Some aspects of early human development Am J Obst & Gynec 44 973-83

199 —— 1944 Information regarding the time of human ovulation derived from a study of 3 unfertilized and 11 fertilized ova Am J Obst & Gynec 47 343-56

200 Rock J Pincus G and Garcia C R 1956 Effects of certain 19 nor steroids on the normal human menstrual cycle Science 124 891-3

117 Hertig A T 1944 The aging ovary—a preliminary note J Clin Endocrin 4 581 2
118 —— 1957 Personal communication
119 Hertig A T Rock J and Adams E C 1956 A description of 34 human ova within the first 17 days of development Am J Anat 98 435 93
120 Hisaw F L 1947 Development of the Graafian follicle and ovulation Physiol Rev 27 95 119
121 Hitschmann F and Adler L 1908 Der Bau der Uterusschleimhaut des geschlechtsreifen Weibes mit besonderer Berucksichtigung der Menstruation Monatsschr f Geburtsh u Gynak 27 1 82
122 Horne H W Jr Nauss J M and Rock J 1950 Unpublished data
123 Huhner M 1913 Sterility in the Male and Female New York Rebman Co
124 Ito H 1938 On the symptoms of ovulation First report Jap J Obst & Gynec 21 9 21
125 —— 1938 On the symptoms of ovulation Second report Jap J Obst & Gynec 21 22 35
126 Jackson M H 1952 Clinical methods of determining ovulation Practitioner 169 151 60
127 Jacobi M P 1877 The Question of Rest for Women During Menstruation The Boylston Prize Essay of Harvard University for 1876 New York G P Putnam s Sons
128 Kalant N Patee C J Simpson G W and Hendelman M 1956 Timing of ovulation Fert & Ster 7 57 65
129 Kaplan I I 1954 Third generation follow up of women treated by x ray therapy for menstrual dysfunction and sterility twenty eight years ago with detailed histories of the grand children born to these women Am J Obst & Gynec 67 484 90
130 Keating F R Jr and Albert A 1949 The metabolism of iodine in Man as disclosed with the use of radioiodine *In* Recent Progress in Hormone Research The Proceedings of the Laurentian Hormone Conference Edited by G Pincus Vol IV New York Academic Press Inc Pp 429 81
131 Kelly J V and Rock J 1956 Culdoscopy for diagnosis in infertility Am J Obst & Gynec 72 523 7
132 Klaften E M 1944 Utero thermometry Study of uterine temperature during reproductive life menopause and amenorrhea J Clin Endocrin 4 159 65
133 Kleegman S J 1954 Therapeutic donor insemination Fert & Ster 5 7 31
134 Knaus H 1935 Die Periodische Fruchtbarkeit und Unfruchtbarkeit des Weibes Der Weg zur Naturlichen Geburtenregelung Second Edition Vienna Wilhelm Maudrich
135 Kraus S D 1947 Observations on the mechanism of ovulation in the frog hen and rabbit West J Surg Obst & Gynec 55 424 37
136 Kroger W S and Freed S C 1951 Psychosomatic Gynecology Including Problems of Obstetrical Care Philadelphia and London W B Saunders Co P 256
137 Krohn P L 1949 Intermenstrual pain (the Mittelschmerz) and the time of ovulation Brit Med J 1 803 5
138 Kundrat H and Engelmann G J 1873 Cited by Ricci J V 1945 *In* One Hundred Years of Gynaecology 1800 1900 Philadelphia The Blakiston Co P 513
139 Kupperman H S Epstein J A Blatt M H G and Stone A 1958 The diagnostic and therapeutic use of intravenous estrogen in anovulation Fert & Ster 9 26 36
140 Lamar J K Shettles L B and Delfs E 1940 Cyclic penetrability of human cervical mucus to spermatozoa in vitro Am J Physiol 129 234 41
141 Landesman R Douglas R G Dreishpoon G and Holze E 1953 The vascular bed of the bulbar conjunctiva in the normal menstrual cycle Am J Obst & Gynec 66 988 98
142 Lang W R 1955 Vaginal acidity and pH A Review Obst & Gynec Surv 10 546 60
143 Laurence P A and Sobel A E 1953 Changes in serum vitamin A level during the human menstrual cycle J Clin Endocrin & Metab 13 1192 200
144 Levin L Buxton C L and Engle E T 1949 On the validity of the hyperemia method for determining ovulation time in women Am J Obst & Gynec 58 795 8
145 Long J A and Evans H M 1922 The oestrous cycle in the rat and its associated phenomena Mem Univ Calif 6 148 pp
146 Macdonald P A and Margolese M S 1950 Luminescent phenomena of the external female genitalia Fert & Ster 1 26 32
147 Mack H C 1943 The glycogen index in the menopause A study of certain estrogen functions based on a new method of staining vaginal smears Am J Obst & Gynec 45 402 19
148 Mandl A M and Zuckerman S 1950 The numbers of normal and atretic ova in the mature rat J Endocrin 6 426 35
149 Margolese M S 1948 Fluorescent phenomena of the vulva associated with sex hormone metabolism J Clin Endocrin 8 603
150 Marshall F H A and Verney E B 1936 The occurrence of ovulation and pseudo pregnancy in the rabbit as a result of central nervous stimulation J Physiol 86 327 36
151 Martin P L 1943 Detection of ovulation by basal temperature curve with correlating endometrial studies Am J Obst & Gynec 46 53-62
152 McCance R A Luff M C and Widdowson E E 1937 Physical and emotional periodicity in women J Hyg 37 571 611
153 McKay D G 1957 Personal communication
154 McKay D G Hertig A T Adams E C and Danziger S 1953 Histochemical observations on the germ cells of human embryos Anat Rec 117 201 19
155 McKay D G and Robinson D 1947 Observations on the fluorescence birefringence and histochemistry of the human ovary during the menstrual cycle Endocrinology 41 378 94
156 McSweeney D J and Wood F O 1940 Acute abdominal conditions following ovulation and its sequelae New Eng J Med 222 174 9
157 Millman N and Hartman C G 1956 Oral control of conception A contemporary survey Fert & Ster 7 110 22
158 Mitchell G W Jr and Rogers J 1953 The influence of weight reduction on amenorrhea in obese women New Eng J Med 249 835 7
159 Moore C R and Wang H 1947 Ovarian activity in mammals subsequent to chemical injury of cortex Physiol Zool 20 300 321
160 Moricard R 1946 Les mécanismes de l ovulation et la standardisation des gonadotrophines *In* Les Troubles de l Ovulation I Rapports 10e Congrè

244 Simkins C S 1932 Development of the human ovary from birth to sexual maturity Am J Anat. 51 465-505
245 Simpson M E and Evans H M 1928 Occurrence of faint bleeding on a definite intermenstrual day in Man Science 68 453
246 Sims J M 1866 Clinical Notes on Uterine Surgery with Special Reference to the Management of the Sterile Condition New York William Wood and Company
247 Smith G V 1955 The ovaries Chapter 6 in Textbook of Endocrinology Second Edition Edited by R H Williams Philadelphia and London W B Saunders Company Pp 350-402
248 Smith G V S and Smith O W 1936 The urinary excretion of estrogenic and gonadotropic hormones during menstrual cycles the period of conception and early pregnancy New Eng J Med 215 908 14
249 —— 1937 Female sex hormones—research Med Rec & Ann 31 101 8
250 Smith G V S Smith O W and Pincus G 1938 Total urinary estrogen estrone and estriol during a menstrual cycle and a pregnancy Am J Physiol 121 98-106
251 Smith J T 1937 Rupture of Graafian follicles Am J Obst & Gynec 33 820 27
252 —— 1938 Rupture of the Graafian follicles II Am J Obst & Gynec 36 455-60
253 Snodgrass J M 1940 Factors influencing bioelectric skin potentials Am J Physiol 129 467
254 Snodgrass J M Rock J and Menkin M F 1943 The validity of ovulation potentials Am J Physiol 140 394-415
255 Snyder F F 1924 Changes in the human oviduct during the menstrual cycle and pregnancy Johns Hopkins Hosp Bull 35 141 6
256 Sommers S C 1958 The Pituitary and Hypothalamus Chapter V in The Endocrinology of Reproduction Edited by Joseph T Velardo New York, Oxford University Press Pp 59 97
257 Squire W 1868 Puerperal temperatures Trans Obst Soc London 9 129-44
258 Stein I F 1943 Gynecography x ray diagnosis in gynecology Surgical Clinics of North America (Chicago Number) Pp 165-80
259 —— 1956 Ultimate results of bilateral ovarian wedge resection Twenty five years follow up Internat J Fert 1 333-44
260 Stein I F and Cohen M R 1950 Sperm survival at estimated ovulation time prognostic significance Fert & Ster 1 169 75
261 Stein I F and Leventhal M L 1935 Amenorrhea associated with bilateral polycystic ovaries Am J Obst & Gynec 29 181 91
262 Steinberg W 1955 Cervical aspects in sterility and infertility Fert & Ster 6 169 79
263 Sternberg W H 1949 The morphology androgenic function hyperplasia and tumors of the human ovarian hilus cells Am J Path 25 493 521
264 Stieve H 1943 Cited by Watzka M 1957 Weibliche Genitalorgane Das Ovarium In Handbuch der Mikroskopischen Anatomie des Menschen Berlin Gottingen Heidelberg Springer Verlag P 5_
265 Stockard C R and P Papanicolaou G N 1917 The existence of a typical oestrous cycle in the guinea pig—with a study of its histological and physiological changes Am J Anat 2_ _25-83
266 Strassmann E O 1941 The theca cone and its tropism toward the ovarian surface a typical feature of growing human and mammalian follicles Am J Obst & Gynec 41 363-78
267 Sturgis S H 1950 The mechanism and control of primate ovulation Fert & Ster 1 40-52
268 Sturgis S H and Pommerenke W T 1950 The clinical signs of ovulation A survey of opinions Fert & Ster 1 113-22
269 Taylor E S and McCallin P F 1952 A correlation of the cytology of the urinary sediment with endometrial histology Am J Obst & Gynec 63 1009-18
270 Tietze C Poliakoff S R and Rock J 1951 The clinical effectiveness of the rhythm method of contraception Fert & Ster 2 444-50
271 Tinklepaugh O L 1933 The nature of periods of sex desire in woman and their relation to ovulation Am J Obst & Gynec 26 335-45
272 Tjio J H and Levan A 1956 Cited in News of Science Science 124 576
273 Tompkins P 1944 The use of basal temperature graphs in determining the date of ovulation. J Am Med A 124 698-700
274 Umbaugh R E 1951 Superovulation and ovum transfer in cattle Fert & Ster 2 243-52
275 Urdan B E and Kurzon A M 1955 Cyclic changes of cervical mucus Obst & Gynec 5 3-12
276 Van de Velde T H 1904 Cited by Barton D S (16)
277 —— 1928 Die Vollkommene Ehe Eine Studie Über Ihre Physiologie und Technik 21st Edition Leipzig and Stuttgart Benno Konegen Medizinischer Verlag P 90
278 — — Ibid Pp 91 93
279 van Horne J 1668 Cited by Adelmann H B 1933 The De Ovorum Gallinaceorum Generationis Primo Exordio Progressuque et Pulli Gallinacei Creationis Ordine of Volcher Coiter Ann Med Hist 5 327-41 444 57
280 van Wagenen G 1934 The incidence of ovulation in the monkey with one ovary Anat Rec 58 (No 4 and Suppl) 91
281 van Wagenen G and Morse A H 1937 Frequency and position of ovulation in the monkey ovary Am J Physiol 119 416 17
282 Venning E H and Browne J S L 1937 Studies on corpus luteum function I The urinary excretion of sodium pregnanediol glucuronidate in the human menstrual cycle Endocrinology 21 711 21
283 Viergiver E and Pommerenke W T 1944 Measurement of the cyclic variations in the quantity of cervical mucus and its correlation with basal temperature Am J Obst & Gynec 48 321 8
284 Vincent W S and Dornfeld E J 1948 Localization and role of nucleic acid in the developing rat ovary Am J Anat 83 437-69
285 von Baer K E 1827 De Ovi Mammalium et Hominis Genesi Cited by Adelmann H B 1933 The De Ovorum Gallinaceorum Generationis Primo Exordio Progressuque et Pulli Gallinacei Creationis Ordine of Volcher Coiter Ann Med Hist. N S 5 327-41 444 57
286 Waldeyer W 1870 Eierstock und Ei Ein Beitrag zur Anatomie und Entwicklungsgeschichte der Sexualorgane Leipzig Verlag von Wilhelm Engelmann
287 Walton A and Hammond J 1928 Observations on ovulation in the rabbit. Brit J Exper Biol 6 190-204
288 Watzka M 1957 Weibliche Genitalorgane Das Ovarium In Handbuch der Mikroskopischen Anat

201 Rock J Reboul J and Snodgrass J M 1938 Electrical changes associated with human ovulation Am J Obst & Gynec 36 733 46
202 Rock J Reboul J and Wiggers H C 1937 The detection and measurement of the electric concomitant of human ovulation by use of the vacuum tube potentiometer New Eng J Med 217 654 8
203 Rogers J 1955 Clinical significance of pregnanediol excretion Fert & Ster 6 513 22
204 —— 1957 Disorders of menstruation In Disease A Month Series (April) Chicago Ill The Year Book Publishers Inc
205 Roland M 1952 A simple test for the determination of ovulation estrogen activity and early pregnancy using the cervical mucus secretion Am J Obst & Gynec 63 81 9
206 Romberg G H 1955 Endometrial aspiration smears in the study of infertility Further observations Fert & Ster 6 302 19
207 Rubenstein B B 1937 The relation of cyclic changes in human vaginal smears to body temperatures and basal metabolic rates Am J Physiol 119 635-41
208 —— 1938 Estimation of ovarian activity by the consecutive day study of basal body temperature and basal metabolic rate Endocrinology 22 41 4
209 —— 1939 Functional sterility in women Ohio State Med J 35 1066 8
210 —— 1940 The vaginal smear—basal body temperature technic and its application to the study of functional sterility in women Endocrinology 27 843 56
211 —— 1951 An emotional factor in infertility A psychosomatic approach Fert & Ster 2 80 6
212 Rubenstein B B and Lindsley D B 1937 Relation between human vaginal smears and body temperatures Proc Soc Exper Biol & Med 35 618 19
213 Rubin I C 1939 The influence of the hormonal activity of the ovaries upon the character of tubal contractions as determined by uterine insufflation A clinical study Am J Obst & Gynec 37 394 404
214 Rydberg E 1948 Observations on the crystallization of the cervical mucus Acta Obst et Gynec Scandinav 28 172 87
215 Sabin M and Latour J P A 1954 A test for the rapid diagnosis of secretory endometrium Fert & Ster 5 44 51
216 Sauramo H 1954 Histology and function of the ovary from the embryonic period to the fertile age Acta Obst et Gynec Scandinav 33 (Suppl 2) 1 25
217 —— 1954 Histology and function of the ovary during the fertile period with special reference to the effect of pregnancy Acta Obst et Gynec Scandinav 33 (Suppl 2) 85 97
218 —— 1954 Attempt at an ontogenetic histological and histopathological synthesis of the functions of the ovary and of the rest of the reproductive system Acta Obst et Gynec Scandinav 33 (Suppl 2) 143 63
219 Sawyer C H Markee J E and Hollinshead W H 1947 Inhibition of ovulation in the rabbit by the adrenergic blocking agent Dibenamine Endocrinology 41 395 402
220 Sax K 1955 Standing Room Only The Challenge of Overpopulation Boston Beacon Press
221 Schroder R 1930 Weibliche Genitalorgane In Handbuch der Mikroskopischen Anatomie des Menschen Bd VII/1 Berlin Julius Springer Pp 329 556
222 Schwarz O H and Young C C Jr 1950 The structure and function of the cortex of the human ovary Am J Obst & Gynec 59 820 30
223 Schwarz O H Young C C Jr and Crouse J C 1949 Ovogenesis in the adult human ovary Am J Obst & Gynec 58 54 64
224 Scott R B 1956 Thickening of the ovarian tunica Effect of its production artificially upon ovulation in monkeys Fert & Ster 7 44 56
225 Seckinger D L and Snyder F F 1926 Cyclic changes in the spontaneous contractions of the human Fallopian tube Johns Hopkins Hosp Bull 39 371 8
226 Seguy J and Simonnet H 1933 Recherche de signes directs d ovulation chez la femme Gynec et Obst 28 657 63
227 Seguy J and Vimeux J 1933 Contribution à l etude des sterilites inexpliquees etude de l ascension des spermatozoides dans les voies genitales basses de la femme Gynec et Obst 27 346-58
228 Seidlin S M 1949 Radioiodine as a diagnostic and therapeutic tool in clinical medicine In Recent Progress in Hormone Research The Proceedings of the Laurentian Hormone Conference Vol IV Edited by G Pincus New York Academic Press Inc Pp 483 510
229 Seward G H 1934 The female sex rhythm Psychol Bull 31 153 92
230 Sharman A 1951 Ovulation after pregnancy Fert & Ster 2 371 93
231 Shaw W 1925 The fate of the Graafian follicle in the human ovary J Obst & Gynaec Brit Emp 32 679 89
232 —— 1948 Textbook of Gynaecology London J and A Churchill Ltd Pp 42 6
233 Shettles L B 1953 Observations on human follicular and tubal ova Am J Obst & Gynec 66 235 47
234 —— 1954 Studies on living human ova Trans N Y Acad Sci Ser II 17 99 102
235 —— 1956 Current Reviews The ovum in infertility abortion and developmental anomaly Fert & Ster 7 561 72
236 Shorr E 1940 A new technic for staining vaginal smears Science 91 321 2
237 —— 1940 A new technic for staining vaginal smears II Science 91 579 80
238 —— 1941 A new technic for staining vaginal smears III a single differential stain Science 94 545 6
239 Shorr E Bernheim A R and Taussky H 1942 The relation of urinary citric acid excretion to the menstrual cycle and the steroidal reproductive hormones Science 95 606 7
240 Siebke H 1930 Ergebnisse von Mengenbestimmungen des Sexualhormons 2 Mitteilung Sexualhormon im Harn bei regelmassigem mensuellem Zyklus Zentralbl f Gynak 54 1601 18
241 Siebke H and Schuschania P 1930 Ergebnisse von Mengenbestimmungen des Sexualhormons 4 Mitteilung Sexualhormon im Harn und Kot bei regelmassigem mensuellem Zyklus Zyklusstorungen und bei Hormontherapie Zentralbl f Gynak 54 1734-47
242 Siegler S L 1944 Fertility in Women Causes Diagnosis and Treatment of Impaired Fertility Philadelphia London Montreal J B Lippincott Company Pp 192 4
243 —— 1952 Treatment of sterility resulting from anovulatory menstruation Fert & Ster 3 150-99

and maturation followed by degenerative changes affecting all layers of the venous wall

In an exhaustive study of the uterine vasculature of the macaque Bartelmez was unable to demonstrate arteriovenous anastomoses in the endometrium [16] This is in agreement with the observations of both Bartelmez [13 1] and Hasner [80] on the vasculature of the human uterus More recently Heckel and Tobin have reported finding arteriovenous anastomoses in the endometria of some but not all of a series of human uteri studied [8]

According to Hirsch and Martin who studied the distribution of nerves in the adult human uterus large nerve trunks enter the body of the uterus from below and laterally They extend along the branches of the uterine artery then pass through the muscle and join other arterial branches dividing eventually into smaller trunks which are distributed in a similar pattern In the innermost one fourth of the myometrium the nerve trunks connect with a series of radial arteries passing through the myometrium between vessels Contact is made with the radial arteries before these vessels divide to give rise to the endometrial spiral and basal arteries Some nerves also connect with the basal arteries and some large trunks extend into the endometrium The nerve trunks contain nonmyelinated or vasomotor and myelinated or sensory fibers Sensory corpuscles of the Vater Pacinian variety were found in crevices of the muscle tissue and in the adventitia of branches of the uterine artery [86]

Okkels also stated that the myometrial nerve plexuses extend into the endometrium following the spiral arteries for a short distance into the functional layer [1 9] Pribor found that the blood vessels of the uterus of the newborn human female are abundantly innervated Some fibers pass within a few cell layers of the epithelium but none was observed to enter the epithelium [89]

Little is known concerning the lymphatics of the endometrium In a single nongravid monkey uterus Wislocki and Dempsey found conspicuous lymphatic networks in both the endometrium and myometrium There was a dense plexus of lymph vessels at the myo-endometrial junction and channels forming characteristic arcades in the deeper two thirds of the mucosa The most superficial layer of the endometrium was however devoid of lymphatics A similar situation was found in a uterus from a monkey pregnant about two months Lymphatics were lacking in the portion of the decidua to which the fetal placenta attaches but were abundant in the deeper half of the decidua In the latter region they tended to congregate in the loose adventitial sheaths of the endometrial spiral arteries The lymphatics throughout the pregnant uterus were greatly enlarged though probably not increased in number [19]

The endometrial vascular bed is not static It is constantly changing throughout the period of sexual maturity Indeed the rise and fall of the endometrial tide is the fundamental feature of the cyclic activity in this tissue

ENDOMETRIAL CYCLIC ACTIVITY

The pattern of the endometrial cyclic activity is alternating phases of growth and regression. The growth phase is a preparation for nidation of the embryo anticipated in the current cycle and the phase of regression is the first stage in preparation for the next anticipated gestation The individual growth phase is normally subject to major variations dependent upon ovulation and fertilization It can be terminated at any functional level But it is always followed by a phase of regression, whatever the level of growth attained This is true of all mammals

Regression results in restoration of the structural functional status characteristic of the nadir of the cyclic excursion In the functional sense this is constant for the species Other aspects of the cyclic activity which are for all practical purposes constant for the species include (1) the duration of the interval between ovulation and nidation (2) the linear distance between implantation site and endometrial basalis or spatial relationship between maternal and fetal circulatory axes and (3) the synchronization of ovum follicle corpus luteum endometrial development which is the fundamental feature of the cyclic activity in all species of mammals Endometrial growth and regression are reciprocal mechanisms acting in sequence to maintain these constant aspects of the cyclic reproductive function These processes are controlled by a complex of reciprocal neural hormonal vascular mechanisms acting in parallel in sequence or in reverse as the case may be This is true of all mammals

Menstruation is an integral feature of the phase of regression in the species in which it occurs It is a specific vascular growth limiting mechanism superimposed upon and acting in conjunction with the more basic mechanisms which control regression in all species of mammals In the human and the macaque it is associated with a relatively marked degree of endometrial vascular growth during the prenidatory stages of the cyclic activity

Ovulation is not an essential prerequisite for menstruation Anovulatory menstrual cycles occur in both the macaque [3] and the human [1 5 194] and in either species they may be normal or abnormal depending on the circumstances With respect to the growth phase there are certain definitive differences between the ovulatory and the anovulatory cycle In the phase of regression, the only differences appear to be of a quantitative nature and these are related

201 Rock J Reboul J and Snodgrass J M 1938 Electrical changes associated with human ovulation Am J Obst & Gynec 36 733 46
202 Rock J Reboul J and Wiggers H C 1937 The detection and measurement of the electric concomitant of human ovulation by use of the vacuum tube potentiometer New Eng J Med 217 654 8
203 Rogers J 1955 Clinical significance of pregnanediol excretion Fert & Ster 6 513 22
204 —— 1957 *Disorders of menstruation In Disease A Month Series (April) Chicago Ill The Year Book Publishers Inc*
205 Roland M 1952 A simple test for the determination of ovulation estrogen activity and early pregnancy using the cervical mucus secretion Am J Obst & Gynec 63 81 9
206 Romberg G H 1955 Endometrial aspiration smears in the study of infertility Further observations Fert & Ster 6 302 19
207 Rubenstein B B 1937 The relation of cyclic changes in human vaginal smears to body temperatures and basal metabolic rates Am J Physiol 119 635 41
208 —— 1938 Estimation of ovarian activity by the consecutive day study of basal body temperature and basal metabolic rate Endocrinology 22 41 4
209 —— 1939 Functional sterility in women Ohio State Med J 35 1066 8
210 —— 1940 The vaginal smear—basal body temperature technic and its application to the study of functional sterility in women Endocrinology 27 843 56
211 —— 1951 An emotional factor in infertility A psychosomatic approach Fert & Ster 2 80 6
212 Rubenstein B B and Lindsley D B 1937 Relation between human vaginal smears and body temperatures Proc Soc Exper Biol & Med 35 618 19
213 Rubin I C 1939 The influence of the hormonal activity of the ovaries upon the character of tubal contractions as determined by uterine *insufflation A clinical study Am J Obst & Gynec* 37 394 404
214 Rydberg E 1948 Observations on the crystallization of the cervical mucus Acta Obst et Gynec Scandinav 28 172 87
215 Sabin M and Latour J P A 1954 A test for the rapid diagnosis of secretory endometrium Fert & Ster 5 41 51
216 Sauramo H 1954 Histology and function of the ovary from the embryonic period to the fertile age *Acta Obst et Gynec Scandinav* 33 (*Suppl* 2) 1 25
217 —— 1954 Histology and function of the ovary during the fertile period with special reference to the effect of pregnancy Acta Obst et Gynec Scandinav 33 (Suppl 2) 85 97
218 —— 1954 Attempt at an ontogenetic histological and histopathological synthesis of the functions of the ovary and of the rest of the reproductive system Acta Obst et Gynec Scandinav 33 (Suppl 2) 143 63
219 Sawyer C H Markee J E and Hollinshead W H 1947 Inhibition of ovulation in the rabbit by the adrenergic blocking agent Dibenamine Endocrinology 41 395-402
220 Sax K 1955 Standing Room Only The Challenge of Overpopulation Boston Beacon Press
221 Schroder R 1930 Weibliche Genitalorgane *In Handbuch der Mikroskopischen Anatomie des Menschen* Bd VII/1 Berlin Julius Springer Pp 329 556

to quantitative aspects of growth rather than to the occurrence of ovulation [110]

In the human and the macaque the pregnant and nonpregnant cycles diverge at the stage of nidation In the nonpregnant cycle the transition from growth to regression occurs approximately 9 12 days after *ovulation i e some 2 5 days after the nidatory level* of function has been attained [1 5 110] Menstruation begins some 4 ± 2 days after the transition from growth to regression and it ceases at a variable interval before the beginning of the next growth phase [110] In other words the menstrual cycle is not synchronous with the growth regression cycle which is the functional unit of the cyclic activity in the endometrium It is customary however for reasons of convenience to use the menstrual cycle as the descriptive unit and the onset of menstruation as the phenomenon of reference in seriating the successive phenomena characteristic of the cyclic activity

In the monkey a phase of rest intervenes between the end of regression and beginning of the next growth phase This is a period of relative equilibrium during which the circulatory activity is stabilized at the basal level It may vary in duration from 1 to 10 days [110] or even longer [15 15] The pattern of variation in the duration of the menstrual cycle in the human suggests a similar situation in this species [104 1 14 33]

Whether the cycle be ovulatory or anovulatory the *growth phase is subdivided into two sub phases* designated by Markee as the primary and secondary periods of growth [110] In the ovulatory cycle these are synchronous respectively with the preovulatory and postovulatory or proliferative and secretory phases

The basic change characteristic of the growth phase is a progressive increase in the volume of blood delivered to and circulating within the endometrial functionalis or nidatory area (Figs 1 5) In the ovulatory cycle the excursion represents an increase from a level which will not support an embryo to a level which is adequate to meet the embryonic requirement at the nidatory stage of its development The change in the volume of circulating blood is paralleled by a progressive increase in the total volume of extravascular fluid and characteristic changes in the relative proportions of water and solids [120 177] which are correlated with changes *in capillary permeability* [81] *and rate of flow in the* stromal capillaries [113]

Of equal importance is vascular growth in particular growth of the spiral arteries which are the sole supply to the nidatory area and which in the event of pregnancy *become placental arteries* [41 140] This increase in functional vascular channel volume is secondary to the increase in the volume of circulating blood It implies a relatively marked difference between basal and nidational blood volume requirements in the species under discussion

Correlated with the changes in circulatory activity is the well known pattern of sequential growth and secretory activity evidenced by the histologic changes demonstrable in fixed sections These progressive changes present characteristic features definitive of the preovulatory and postovulatory phases respectively (Figs 13 24) In anovulatory cycles the nidatory level of function is not attained and the morphologic characteristics definitive of the post ovulatory phase are lacking

The third major type of cyclic activity in the endometrium is at the biochemical level Qualitative and quantitative changes in the metabolic and enzymatic activity can be *correlated with the cyclic* vascular changes on the one hand and with the changing aspects of function in the endometrial cells on the other The metabolic activity minimal at the basal level of the cyclic excursion reaches its peak at about the time of arrival of the ovum in the uterus and remains high until after the transition from growth to regression [118 73 187]

The activity of the types described results in a progressive increase in the thickness and volume of the endometrium (Figs 1 5) The area of intraocular transplants increases progressively to mid cycle at which time there is a slight and temporary decrease of some 2 4 days duration This occurs *in both ovulatory and anovulatory cycles* in the former it is related chronologically to ovulation Following this temporary decrease the progressive increase is resumed and it continues until about five days before the onset of menstruation Regression becomes evident about *24 hours after growth* ceases The transplant approximately doubles in area in each of the two subdivisions of the growth phase [110] In the human the endometrium increases in thickness from about 1 to 5 mm the excursion being subject to minor variation

Biochemical data on quantitative aspects of growth in the human endometrium have been reported recently by McLennan and Koets [1 0] Analyzing comparable specimens of endometrium removed on successive days of the menstrual cycle these investigators found a progressive increase in total water and solids up to the stage of nidation Their data indicated a 3 8 fold increase in total wet weight a 3 6 fold increase in total water and a 5 7 fold increase in total protein on day 22 as compared with day 9 of the menstrual cycle

The percentage of water increases rapidly in the early stages of the growth phase is highest at the *midproliferative* stage (about day 10) drops to a somewhat lower level as solids are laid down in the postovulatory phase and decreases rapidly during regression to reach a minimum during menstrua

tion Conversely the percentage of solids is lowest at the midproliferative stage higher in the post ovulatory phase and highest during menstruation [120] A similar pattern of variation in the monkey was demonstrated by Van Dyke and Ch en [177 178]

The changes in water and solids run *pari passu* with changes in the activity of the capillaries The growth phase is initiated by an increase in the num ber of capillaries carrying blood to the stroma and in the rate of flow in these vessels The increase continues to a maximum late in the proliferative phase After ovulation there is a decrease to a some what lower level which is maintained through the stage of nidation then a rapid decrease from the 96th to the 48th hour before the onset of menstrua tion [111]

Both ovarian hormones influence the activity of the endometrial capillaries but estrogen has rela tively the greater effect on those supplying the stroma Progesterone on the other hand appears to play a relatively greater part in controlling the ac tivity of the subepithelial and periglandular capil laries [118]

CYCLIC VASCULAR CHANGES

The cyclic changes in the spiral arteries of the macaque first demonstrated by Daron [1] have been studied by means of reconstructions by both *Daron* [41] *and Bartelmez* [18] *They were studied in* intra-ocular endometrial transplants by Markee [110] and Phelps [12] [7] and in routine histologic prepara tions by Kaiser [89] They have been produced experi mentally in oophorectomized monkeys in response to estrogen and progesterone administered in a time pattern simulating the natural cycle [1 7] By injecting progesterone alone in oophorectomized monkeys Hartman and Speert obtained arterial growth into the nidatory area and withdrawal bleeding as well But with this treatment the arteries were poorly developed structurally [77]

Studies on the cyclic vascular activity in the hu man endometrium include those of Saito [24] Bartel mez [18] Jones and Brewer [9] Hasner [6] and Okkels [1 6] Also pertinent are Ramsey s observations on the vas cular activity characteristic of pregnancy in both the human being [4] and the Macaque [4 41]

The cyclic vascular activity as observed in intra ocular endometrial transplants is illustrated in Fig ures 1 11 During the growth phase of the cycle the spiral arteries exhibit progressive lengthening tor tuosity and certain distinctive changes in the topo graphical relationships between arteries and endo metrial surface respectively [41 89 110 137] At the begin ning of the growth phase these arteries extend about halfway through the endometrium Long ar terioles arising at their distal ends pass to the subepithelial capillary plexus As the cycle pro gresses the distal ends of the arteries approach nearer and nearer the surface so that at the stage of nidation they extend almost to the surface their terminal branches occupying a narrow zone sub-jacent to the surface epithelium (Figs 1 5 and 12) The arteries lengthen more rapidly than the endo metrium thickens As a result the arteries become progressively more coiled Meanwhile adventitia forms a column and together with its surrounding connective tissue encloses the coiled artery Marked coiling and complexity of the spirals is characteristic of the postovulatory phase These features are lack ing in anovulatory cycles and the arterial pattern at the onset of bleeding is comparable to that of the proliferative phase of the ovulatory cycle (Figs 10 and 11) Moreover considerable regression of arteries developed in ovulatory cycles may take place during subsequent anovulatory cycles [8]

In fixed tissues the spiral arteries appear as longi tudinal columns or arterial fields (cf Fig 12) At the beginning of the growth phase the typical field is comprised of 6 8 loops An increase to about 12 loops may be observed in an anovulatory cycle But with the formation of a corpus luteum lengthening of the arteries is more rapid and within a week after ovulation the typical field contains 12 15 loops [89] Kaiser observed that the spiral arteries ex hibit an area of maximal coiling and cyclic changes *in the position of this area in relation to the endo-* metrial basalis At the time of follicular maturity arterial fields were found only in the basal one third of the endometrium Under the influence of pro gesterone the fields move further and further out until eventually they are found in the superficial half with smaller fields located just under the sur face epithelium [89] At the stage of nidation the ar terial coils can sometimes be seen bulging the sur face of intra ocular endometrial transplants (Fig 5) [137]

Kaiser found little variation in the arterial fields in any one endometrium In particular the relative *position of the arterial fields* with respect to the myometrium was remarkably constant in all sec tions and all areas of each section from any one animal This suggests synchronization of the ac tivity of different arteries during the growth phase at least with respect to control of the changing topographical relationships of the area of maximal coiling

In studying fixed sections of macaque endome trium Kaiser noted histologic changes in the walls of the coiled arteries that were correlated with and proportional in degree to progestational alterations of the epithelium and stroma [8]

The disproportionate growth of the spiral arteries the marked coiling which accommodates the arter

to quantitative aspects of growth rather than to the occurrence of ovulation [110]

In the human and the macaque the pregnant and nonpregnant cycles diverge at the stage of nidation In the nonpregnant cycle the transition from growth to regression occurs approximately 9 12 days after ovulation i e some 2 5 days after the nidatory level of function has been attained [17, 5, 110] Menstruation begins some 4 ± 2 days after the transition from growth to regression and it ceases at a variable in*terval before the beginning of the next growth* phase [11] In other words the menstrual cycle is not synchronous with the growth regression cycle which is the functional unit of the cyclic activity in the endometrium It is customary however for reasons of convenience to use the menstrual cycle as the descriptive unit and the onset of menstruation as the phenomenon of reference in seriating the successive *phenomena* characteristic of the cyclic activity

In the *monkey* a phase of *rest intervenes* between the end of regression and beginning of the next growth phase This is a period of relative equilibrium during which the circulatory activity is stabilized at the basal level *It may vary* in duration from 1 to 10 days [110] or even longer [15, 161] The pattern of variation in the duration of the menstrual cycle in the human *suggests* a similar situation in this species [1, 4, 17, 14, 33]

Whether the cycle be ovulatory or anovulatory the growth phase is subdivided into two sub phases designated by Markee as the primary and secondary periods of growth [110] In the ovulatory cycle these are synchronous respectively with the preovulatory and postovulatory or proliferative and secretory phases

The basic change characteristic of the growth *phase is a progressive* increase in the volume of blood delivered to and circulating within the endometrial functionalis or nidatory area (Figs 1 5) In the ovulatory cycle the *excursion represents* an increase from a level which will not *support an em*bryo to a level which is adequate to meet the embryonic requirement at the nidatory stage of its development The change in the volume of circulating blood is paralleled by a progressive increase in *the total* volume of extravascular fluid and characteristic changes in the relative proportions of water and solids [120, 177] which are correlated with changes in capillary permeability and rate of flow in the stromal capillaries [113]

Of *equal importance* is vascular growth in particular growth of the *spiral arteries which* are the sole supply to the nidatory *area and which* in the event of pregnancy become *placental arteries* [41, 140] *This increase in functional* vascular channel volume is secondary to the increase in the *volume of cir*culating blood It implies a relatively marked difference between basal and nidational blood volume *requirements in the species under discussion*

Correlated with the *changes in circulatory activity* is the well known pattern of sequential growth and secretory activity evidenced by the histologic changes demonstrable in fixed sections These progressive changes present characteristic features definitive of the preovulatory and postovulatory phases respectively (Figs 13 24) In anovulatory cycles the nidatory level of function is not attained and the *morphologic characteristics definitive of the post* ovulatory phase are lacking

The third major type of cyclic activity in the endometrium is at the biochemical level Qualitative and quantitative changes in the *metabolic and en*zymatic activity can be correlated with the cyclic vascular changes on the one hand and with the changing aspects of function in the endometrial cells on the other The metabolic activity minimal at the basal level of the cyclic excursion reaches its peak at about the time of arrival of the ovum in the uterus and remains high until after the transition from growth to regression [118, 78, 167]

The activity of the types described results in a progressive increase in the thickness and volume of the endometrium (Figs 1 5) The area of intra ocular transplants increases progressively to mid cycle at which time there is a slight and temporary decrease of some 2 4 days *duration* This occurs in both ovulatory and anovulatory cycles *in* the former it is related chronologically *to ovulation* Following this temporary decrease the progressive increase is resumed and it continues until about five days before the onset of menstruation Regression becomes evident about 24 hours after growth ceases The transplant approximately doubles in area in each of the two subdivisions of the growth phase [1] In the human the endometrium increases in thickness from about 1 to 5 mm the excursion being subject to minor variation

Biochemical data on quantitative aspects of growth in the *human endometrium have been reported* recently by McLennan and Koets [120] *Analyzing* comparable specimens of endometrium removed on successive days of the *menstrual cycle these investigators* found a progressive *increase in total water* and solids up to the stage of *nidation Their data* indicated a 3 8 fold *increase in total wet weight* a 3 6 fold increase *in total water and a 5 7 fold in*crease in *total protein on* day 22 as compared with day *9 of the menstrual cycle*

The *percentage* of water increases rapidly in the *early stages of the* growth phase is highest at the midproliferative *stage* (about day 10) drops to a *somewhat lower* level as solids are laid down in the *postovulatory phase* and decreases rapidly during *regression* to reach a minimum during menstrua

tion Conversely the percentage of solids is lowest at the midproliferative stage higher in the post ovulatory phase and highest during menstruation [120] A similar pattern of variation in the monkey was demonstrated by Van Dyke and Chen [177 178]

The changes in water and solids run *pari passu* with changes in the activity of the capillaries The growth phase is initiated by an increase in the number of capillaries carrying blood to the stroma and in the rate of flow in these vessels The increase continues to a maximum late in the proliferative phase After ovulation there is a decrease to a somewhat lower level which is maintained through the stage of nidation then a rapid decrease from the 96th to the 48th hour before the onset of menstruation [118]

Both ovarian hormones influence the activity of the endometrial capillaries but estrogen has relatively the greater effect on those supplying the stroma Progesterone on the other hand appears to play a relatively greater part in controlling the activity of the subepithelial and periglandular capillaries [118]

CYCLIC VASCULAR CHANGES

The cyclic changes in the spiral arteries of the macaque first demonstrated by Daron [41] have been studied by means of reconstructions by both Daron [41] and Bartelmez [16] They were studied in intra ocular endometrial transplants by Markee [1 0] and Phelps [134-7] and in routine histologic preparations by Kaiser [9] They have been produced experimentally in oophorectomized monkeys in response to estrogen and progesterone administered in a time pattern simulating the natural cycle [1 7] By injecting progesterone alone in oophorectomized monkeys Hartman and Speert obtained arterial growth into the nidatory area and withdrawal bleeding as well But with this treatment the arteries were poorly developed structurally [77]

Studies on the cyclic vascular activity in the human endometrium include those of Saito [15] Bartelmez [13] Jones and Brewer [4] Hasner [0] and Okkels [2] Also pertinent are Ramsey s observations on the vascular activity characteristic of pregnancy in both the human being and the Macaque [1 0]

The cyclic vascular activity as observed in intra ocular endometrial transplants is illustrated in Figures I 11 During the growth phase of the cycle the spiral arteries exhibit progressive lengthening tortuosity and certain distinctive changes in the topographical relationships between arteries and endometrial surface respectively [7] At the beginning of the growth phase these arteries extend about halfway through the endometrium Long arterioles arising at their distal ends pass to the subepithelial capillary plexus As the cycle progresses the distal ends of the arteries approach nearer and nearer the surface so that at the stage of nidation they extend almost to the surface their terminal branches occupying a narrow zone subjacent to the surface epithelium (Figs 1 5 and 12) The arteries lengthen more rapidly than the endometrium thickens As a result the arteries become progressively more coiled Meanwhile adventitia forms a column and together with its surrounding connective tissue encloses the coiled artery Marked coiling and complexity of the spirals is characteristic of the postovulatory phase These features are lacking in anovulatory cycles and the arterial pattern at the onset of bleeding is comparable to that of the proliferative phase of the ovulatory cycle (Figs 10 and 11) Moreover considerable regression of arteries developed in ovulatory cycles may take place during subsequent anovulatory cycles [6]

In fixed tissues the spiral arteries appear as longitudinal columns or arterial fields (cf Fig 12) At the beginning of the growth phase the typical field is comprised of 6 8 loops An increase to about 12 loops may be observed in an anovulatory cycle But with the formation of a corpus luteum lengthening of the arteries is more rapid and within a week after ovulation the typical field contains 12 15 loops [99] Kaiser observed that the spiral arteries exhibit an area of maximal coiling and cyclic changes in the position of this area in relation to the endometrial basalis At the time of follicular maturity arterial fields were found only in the basal one third of the endometrium Under the influence of progesterone the fields move further and further out until eventually they are found in the superficial half with smaller fields located just under the surface epithelium [9] At the stage of nidation the arterial coils can sometimes be seen bulging the surface of intra-ocular endometrial transplants (Fig 5) [137]

Kaiser found little variation in the arterial fields in any one endometrium In particular the relative position of the arterial fields with respect to the myometrium was remarkably constant in all sections and all areas of each section from any one animal [9] This suggests synchronization of the activity of different arteries during the growth phase at least with respect to control of the changing topographical relationships of the area of maximal coiling

In studying fixed sections of macaque endometrium Kaiser noted histologic changes in the walls of the coiled arteries that were correlated with and proportional in degree to progestational alterations of the epithelium and stroma [99]

The disproportionate growth of the spiral arteries the marked coiling which accommodates the arter

to quantitative aspects of growth rather than to the occurrence of ovulation [110]

In the human and the macaque the pregnant and nonpregnant cycles diverge at the stage of nidation In the nonpregnant cycle the transition from growth to regression occurs approximately 9 12 days after ovulation i e some 2 5 days after the nidatory level *of function has been attained* [17, 110] Menstruation begins some 4 ± 2 days after the transition from growth to regression and it ceases at a variable interval before the beginning of the next growth phase [110] In other words the menstrual cycle is not synchronous with the growth regression cycle which is the functional unit of the cyclic activity in the *endometrium It* is customary however for reasons of convenience to use the menstrual cycle as the descriptive unit and the onset of menstruation as the phenomenon of reference in seriating the successive phenomena characteristic of the cyclic activity

In the monkey a phase of rest intervenes between the end of regression and beginning of the next growth phase This is a period of relative equilibrium during which the circulatory activity is stabilized at the basal level It may vary in duration from 1 to 10 days [1] or even longer [15, 151] The pattern of variation in the duration of the menstrual cycle in the human *suggests* a *similar situation in this species* [1, 4, 1, 14, 33]

Whether the cycle be ovulatory or anovulatory the growth phase is subdivided into two sub phases designated by Markee as the primary and secondary periods of growth [110] In the ovulatory cycle these are synchronous respectively with the preovulatory and postovulatory or proliferative and secretory phases

The basic change characteristic of the growth phase is a progressive increase in the volume of blood delivered to and circulating within the endometrial functionalis or nidatory area (Figs 1 5) In the ovulatory cycle the excursion represents an increase from a level which will not support an embryo to a level which is adequate to meet the embryonic requirement at the nidatory stage of its development The change in the volume of circulating blood *is paralleled by a progressive increase in* the total volume of extravascular fluid and characteristic changes in the relative proportions of water and solids [1, 0, 177] which are correlated with changes in capillary permeability [81] and rate of flow in the stromal capillaries [113]

Of equal importance is vascular growth in particular growth of the spiral arteries which are the sole supply to the nidatory area and which in the *event of pregnancy become* placental *arteries* [41, 140] This increase in functional vascular channel volume is secondary to *the increase in the volume of circulating* blood It implies a relatively marked difference between basal and nidational blood volume requirements in the species under discussion

Correlated with the changes in circulatory activity is the well known pattern of sequential growth and secretory activity evidenced by the histologic *changes demonstrable in fixed sections These* progressive changes present characteristic features definitive of the preovulatory and postovulatory phases respectively (Figs 13 24) In anovulatory cycles the nidatory level of function is not attained and the morphologic characteristics definitive of the post ovulatory phase are lacking

The third major type of cyclic activity in the endometrium is at the biochemical level Qualitative and quantitative changes in the metabolic and enzymatic activity can be correlated with the cyclic vascular changes on the one hand and with the changing aspects of function in the endometrial cells on the other The metabolic activity minimal at the basal level *of the cyclic excursion reaches its* peak at about the time of arrival of the ovum in the uterus and remains high until after the transition from growth to regression [118, 73, 167]

The activity of the types described results in a progressive increase in the thickness and volume of the endometrium (Figs 1 5) The area of intraocular *transplants increases progressively to mid* cycle at which time there is a slight and temporary *decrease of some 2 4 days duration This* occurs in both ovulatory and anovulatory cycles in the former it is related chronologically to ovulation Following this temporary decrease the progressive increase is resumed and it continues until about five days before the onset of menstruation Regression becomes evident about 24 hours after growth *ceases The transplant approximately* doubles in area in each of the two subdivisions *of the growth* phase [2] In the human the endometrium *increases* in thickness from about 1 to 5 mm the excursion being subject to minor variation

Biochemical data on quantitative aspects of growth in the human endometrium have been reported recently by McLennan and Koets [1, 0] Analyzing comparable specimens of endometrium removed on *successive days* of the menstrual cycle these investigators found a progressive increase in total water and solids up *to the stage of nidation Their data* indicated a 3 8 fold increase in total wet weight a 3 6 *fold* increase in total water and a *5 7 fold* increase in total protein on day 22 as compared with day 9 of the menstrual cycle

The percentage of water increases rapidly in the early stages of the growth phase is highest at the midproliferative stage (about day 10) drops to a somewhat lower level as solids are laid down in the postovulatory phase and decreases rapidly during regression to reach a minimum during menstrua

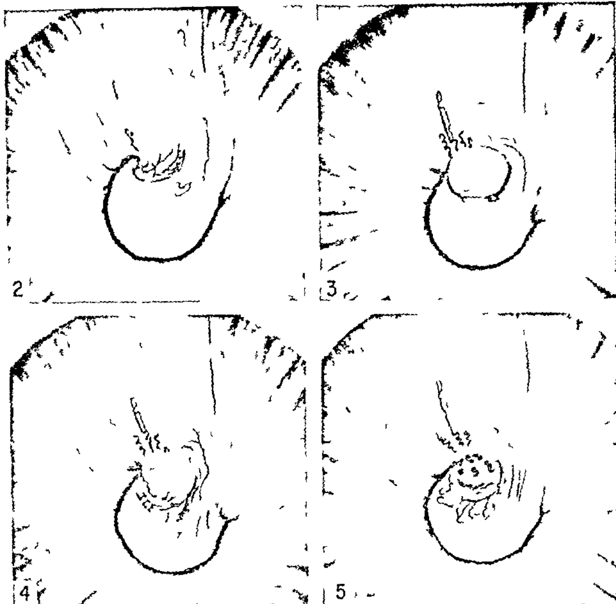


PLATE 1 FIG 2 Day 1 of experimental cycle induced by administration of estrogen progesterone The transplant is in the condition typical of the untreated oophorectomized state—relatively dry grayish white Its surface is just about on a level with that of the bulb iris junction (c in Figure 1)

FIG 3 Day 12 of the same cycle height of the proliferative phase induced by estrogen Growth and increased succulence are evident The bulb has rounded up and protrudes above the level of the bulb iris junction The largest arterial branches now well below the surface are still faintly perceptible but most of the arterial pattern has been obscured as a result of the superficial growth Development of the superficial capillary network and the deep pink color of the bulb are indicated by the fine stippling over its surface The stage illustrated corresponds functionally to the stage of the human cycle illustrated in Figure 15 (Plate 6) and to late proliferative phase illustrated in Plate 16

FIG 4 Day 18 fourth day of progesterone administration The vessels in the bulb are not the same ones shown in preceding figures but branches from the deep network which have grown upward into the superficial layer since administration of progesterone was begun From this point on significant changes include extension of arterioles toward the surface extension into the crescent of branches from the bulb network and thereafter progressive development of the vascular bed in the crescent Stage of the experimental cycle corresponds to stage of natural cycle defined by arrival of ovum in uterus Compare with Figures 17 and 18 (Plates 8 and 9)

FIG 5 Day 22 eighth day of progesterone administration Arterial coils lie just under the surface and are beginning to bulge the surface as a result of shifts in the extravascular fluid and slight shrinkage of the bulb The well-developed arteries impart a red glow to the transplant The coils of artery 10 (Fig 1) are congested and protruding Looping and anastomosis which are characteristic of the response to progesterone are conspicuous in the well developed vascular bed of the crescent There is some tree like branching also Stage of activity illustrated corresponds functionally to the stage of beginning implantation in the fertile cycle or height of the progestational phase of the nonpregnant cycle It is comparable to the stage of the human cycle illustrated in Figures 19 21 (Plates 10 12) and approximately to the progestational stage illustrated in Plate 16 (Reproduced from Phelps Endometrial vascular reactions and the mechanism of nidation American Journal of Anatomy 79 183 5 1946 figs 5 11 13 15)

ies to the limited space and the close approximation of the terminal ends of the arteries and the endometrial surface at the stage of nidation are characteristic of the postovulatory phase of the cycle [41, 99] and of the endometrial response to progesterone [137] Moreover it has been shown experimentally that these changes are related specifically to the time of nidation in relation to the secretion of progesterone [137] Finally in her brilliant study of the vascular changes characteristic of pregnancy in the rhesus monkey Ramsey found that the progressive growth and coiling of the spiral arteries continues during the first 80 or 90 days Then at the time of conversion when stretching supplants intrinsic growth as the mechanism of uterine enlargement [144] the coils of the arteries are payed out very much as the coils of a rope are payed out [140, 142] It is evident therefore that the disproportionate growth and coiling of the arteries which characterize the nonpregnant cycle are essential features of the preparation of the endometrial vascular bed for nidation in the species under discussion and that such preparation requires a relatively marked degree of prenidatory arterial growth Whether the characteristic changes in the topographical relationships of the arteries and endometrial surface are related to any specific requirement of topograpical distribution of blood in the nidatory area remains to be determined Of interest in this connection are Boving s observations on the mechanics and chemistry of attachment of the embryo in the rabbit in particular the observation that the early trophoblast invasion tends to be associated with epithelium over lying a blood vessel [21, 2]

Endometrial regression is the reverse or reciprocal of growth It is a process of circulatory deprivation relative or total as the case may be The characteristic features of this phase of the cyclic activity are (1) a progressive decrease in the volume of blood delivered to and circulating within the function

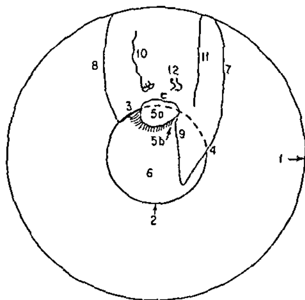


FIG 1 KEY TO FIGURES 2 11 PLATES 1-3 diagrammatic representation of the intra-ocular endometrial transplant pictured in Figures 2 11 as it appeared at the beginning of the cycle illustrated in Figures 2 9

1 *Periphery of iris*

2 Pupil border of iris Dotted line from 3 to 4 reading clockwise indicates that part of the pupil border which lies under and is obscured by regions 5a and 9 of the transplant.

5 The endometrial transplant It consists of a protruding bulbous portion (*a*) and a flat crescent shaped extension (*b*) from the lower surface of the bulbous portion The two parts are referred to hereafter as bulb and crescent respectively Superficially iris and endometrial tissue merge along c

6 A flat film of indifferent endometrial tissue having the characteristics of basalis It is attached to the iris at the pupil margin under 5a and 9 and passes under the pupil margin from 4 to 3 reading clockwise Variations in shading in area 6 indicate variations in texture of the film.

7 8 These are fine brown lines delimiting that portion of the iris occupied by the vascular field from which the transplant is supplied The extent of this vascular field is indicated in Figures 2 11 by the large light area above the transplant Arterial branches ramify throughout this area and extend into 9 Only a few of the superficial twigs are shown in the drawings

Lines 7 and 9 and the pupil margin of the iris lie at the normal iris level The surface of the vascular area enclosed by 7 and 8 is slightly higher rising to a distinct mound just above the transplant in the area occupied by the coils of arteries 10 and 12 Under 5a and for a short distance above 3 the *slope of the mound is precipitous* elsewhere it is gradual in all directions

9 An extension of iris fibers into the pupil area It is attached to the film (6) and to the crescent (5*b*)

10 11 12 Main arterial trunks supplying the transplant Only the superficial portions are shown

EXPLANATION OF FIGURES 2 11 PLATES 1 3

Figures 2 11 are artist s transcriptions of camera lucida drawings of a single homotransplant of endometrium *growing on the iris of the left eye of an oophorectomized* rhesus monkey Figures 2 9 illustrate successive stages in the response of the transplant to a single cycle of stimulation by ovarian hormones in the following dosage 500 I U estrogen daily for 28 days 2 mgm progesterone daily days 15 28 The stages illustrated are comparable to significant functional levels in the natural endometrial cycle

*Figures 10 and 11 illustrate respectively the endome*trial *vascular responses to estrogen progesterone and* estrogen

The original camera lucida drawings were made at a magnification of 12 diameters All blood vessels shown were perceptible with *magnifications* of 25 diameters or less

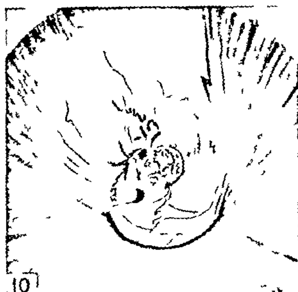




PLATE 3

Fig 10 The intra-ocular endometrial transplant as it appeared on day 22 of another cycle induced by administration of estrogen and progesterone Dosages same as in the cycle illustrated in Figures 2 9 stage same as illustrated in Figure 5 corresponding functionally to beginning implantation in the fertile cycle The development and extension upward of the coiled arteries is so marked that the bulb is fiery red and individual vessels can hardly be distinguished This extension of the arteries into the nidatory area is characteristic of the response to progesterone and does not occur when only estrogen is given (137) This may be seen by comparing Figures 10 and 11

Fig 11 The transplant as it appeared on day 22 of a cycle in which only estrogen was given 500 I U daily The bulb is pale pink It does not protrude above the surface of the iris but slopes rather sharply downward from its high point just below the bulb-iris junction Some of the spiral arteries are perceptible due to the shrinkage of the overlying tissue around them but they are not tightly coiled and there is no arterial development in the superficial area comparable to that shown in Figure 10

Figures 10 and 11 illustrate the characteristic differences in the arterial development in ovulatory and anovulatory cycles respectively

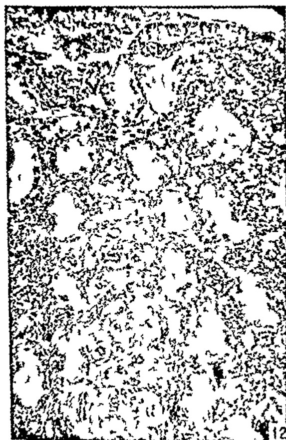


Fig 12 Section of monkey endometrium removed at the end of the progestational phase of a cycle induced by administration of 500 I U of estrogen daily for 28 days 2 mgm progesterone daily days 15 28 Noteworthy are the arterial column formed by the coils of a single spiral artery the approximation of the end of this artery and the endometrial surface and the progestational reaction in the endometrial glands and stroma (× 140) (Figures 10 and 11 reproduced from Phelps Endometrial vascular reactions and the mechanism of nidation American Journal of Anatomy 79 191 fig 32 185 fig 18 1946)

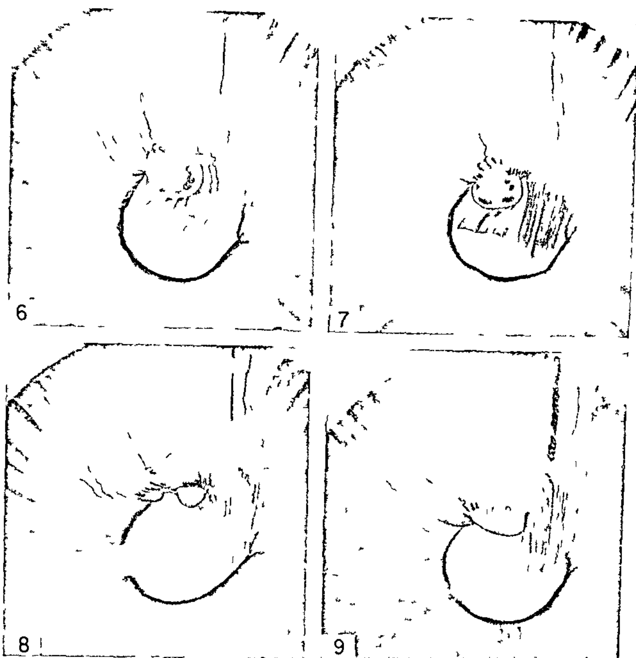


PLATE 2

Fig 6 Day 31 of the experimental cycle day 3 after last injection day 1 of uterine bleeding Shrinkage of the transplant is evident also increased coiling of the arteries Attention is called to the prominent arteriole on the observer's right The overlying tissue has shrunk around it and it bulges the surface throughout The phase of premenstrual regression is illustrated at approximately the onset of the final stage of premenstrual ischemia The corresponding stage in the human cycle is illustrated in Figure 24 (Plate 15)

Fig 7 Day 32 day 4 after last injection day 1 of bleeding from transplant This figure shows hematoma formation in the bulb and hemorrhage in the crescent area which is hemorrhagic throughout Hematomata shown formed and discharged over a period of several hours they did not form simultaneously Later in the day bleeding from hematomata occurred in the crescent area

Dark shading in the iris projection indicates thin walled contiguous vessels which opened up during bleeding and regressed gradually as circulation in the bulb was restored

Fig 8 Day 9 after last injection The bulb has shrunk to its minimum size for the cycle The surface is well below that of the iris Bulb crescent and film (6 in Fig 1) are completely ischemic and the endometrial tissue is dry dirty white scaly In experimental cycles the interval during which the transplant remains at the minimal level of activity is variable

Fig 9 Day 23 after last injection Significant features are increase in the size of the bulb and return of circulation in its vessels regression of vessels in the iris extension and tight coiling of artery 11 (Fig 1) This stage represents rebound from the minimum and is more nearly comparable to normal base than is the extreme degree of regression illustrated in Figure 8 With reference to the relative level of vascular activity it is comparable approximately to the early proliferative stage illustrated in Figure 13 (Plate 4) (Reproduced from Phelps Endometrial vascular reactions and the mechanism of nidation American Journal of Anatomy 79 187 9 1946 figs 19 21 25 27)

plished primarily by new growth connecting the basal portions of the coiled arteries and veins Soon after the necrotic tips of the coiled arteries become thrombosed the arteries relax and blood begins to enter The relaxation like the constriction is gradual and prolonged As it progresses capillaries grow out from the arteries at the middle third of the functional zone These join the veins just to the depth of their collapsed and apparently obliterated ends At first only a few red cells pass through these new endothelial tubes but as the capillary loops lengthen they acquire thicker walls and progress to the order of arterioles The number of spiral arteries salvaged in this manner varies in different cycles and is inversely proportional to the extent of arterial desquamation

Certain arteries which function as basal arteries in one cycle may become typical coiled arteries *in the functional zone during a following cycle*[110] Stages transitional between straight (or basal) and coiled arteries are to be found in all phases of the cycle[14,17] The number of arteries thus transformed *appears to be inversely proportional* to the number of coiled arteries that are regenerated i e directly proportional to the extent of arterial desquamation The latter as already noted is directly proportional to the extent of arterial growth in the preceding growth phase[1,9]

Re epithelialization is accomplished by migration of cells from the mouths of the glands[119,10] It is usually complete within a few hours after termination of hemorrhage in a given area and precedes termination of regression in that area If the duration of a given menstrual period is shortened by administering estrogen or progesterone this result is achieved by hastening revascularization and re establishment of the blood supply to the functional zone The dosage of estrogen required is somewhat smaller than the dose required to initiate growth[110]

The intimate relationship between menstrual bleeding and endometrial regression was demonstrated by Markee in experiments of several different types For example by slowly decreasing the *estrogen level in an oophorectomized monkey* slow regression was induced and this was not accompanied by bleeding But a sudden decrease of sufficient magnitude was followed by rapid regression and bleeding When progesterone was injected following estrogen withdrawal bleeding was prevented if the dosage of progesterone was sufficient to maintain growth But if the dosage was not adequate to prevent regression bleeding occurred In comparing responses to different types of estrogen it was found that when comparable degrees of growth were induced the duration of the interval between onset of endometrial regression and onset of bleeding remained constant whereas the interval between cessation of treatment and onset of bleeding varied according to the type of estrogen administered[11]

By inserting a crystal of estrone into one eye of a monkey bearing intra-ocular endometrial transplants it was possible to vary the local concentration of estrogen independently of the systemic level In such experiments regression and bleeding were prevented in the treated eye while the uterus and transplants in the untreated eye were bleeding Conversely removal of the crystal was followed by regression and bleeding in the treated eye but not in the uterus or transplants in the untreated eye In these experiments bleeding occurred only in transplants that were regressing rapidly and could not be induced in the absence of regression[112] These results indicate that menstrual bleeding can result from purely local changes which lead to endometrial regression The relationship of menstruation *to a reduction in the local blood supply is also in*dicated by the fact that uterine bleeding is precipitated by spinal transection presumably as a result of vascular shock[114,181,98]

It is well known that oophorectomy during the period of sexual maturity is followed by a premature menstrual period[8] Van Wagenen and Aberle found that bleeding did not occur in monkeys if the operation *was performed very early in the cycle*[90] Zuckerman showed that a certain minimal dosage of estrogen is required to produce withdrawal bleeding in oophorectomized monkeys[196,7] and similar results were obtained with progesterone by Eckstein[50] These observations indicate that a minimal degree of endometrial growth is prerequisite for menstrual bleeding Striking evidence of a relationship between quantitative aspects of growth and quantitative aspects of bleeding were obtained by Markee in a study of differences between ovulatory and anovulatory cycles With respect to qualitative aspects of regression the two types of cycles were similar But differences were noted in quantitative aspects of regression bleeding and desquamation both between the two types of cycles and among the cycles of each type These differences were correlated with differences in quantitative aspects of growth rather than with the occurrence or nonoccurrence of ovulation[110] Correlations have also been observed between quantitative aspects of the growth occurring in one cycle *and quantitative aspects of bleeding occurring in* subsequent cycles[136]

CAUSE AND SIGNIFICANCE OF MENSTRUATION

Menstrual bleeding is precipitated by inflow of blood under pressure into arteries which have previously been subjected to the effects of anoxemia dehydration and ischemia for periods of 2 6 days In other

als (2) a parallel decrease in the volume of extra vascular fluid (3) shrinkage and collapse of the intrinsic endometrial structures (4) the menstrual hemorrhage and desquamation and (5) reorganization of the terminal vascular bed and stabilization of the circulatory activity at the basal level These *changes result in a progressive decrease in the endometrial thickness and volume* This decrease continues throughout the period of bleeding and for a short time after cessation of bleeding (Figs 6 8)[110, 137]

Menstruation is not an essential feature of endometrial regression It is an integral feature of the phase of regression in the species in which it occurs It results from the regression and contributes to it *It is invariably preceded by regression and unless* the antecedent regression is rapid and extensive bleeding does not occur[110, 11]

The premenstrual decrease in the volume of an intra ocular endometrial transplant may vary from 26 to 76 per cent The average relative decrease is about 35 per cent before the onset of hemorrhage 60 per cent during bleeding and 5 per cent after cessation of flow *About one fourth of the decrease* during bleeding is due to desquamation three fourths to resorption of tissue and fluid These quantitative data are not necessarily applicable to man[110]

In the monkey the duration of the premenstrual stage of regression is about 4 ± 2 days This stage is initiated by a reduction in blood supply to the functionalis and *in the volume of extravascular fluid* The rate of flow in the capillaries decreases rapidly and there is also a relative decrease in the amount of hemoglobin[111] As the endometrium decreases in thickness the arteries become more and more coiled accommodating themselves thus to the restricted space An artery may develop as many as eight new coils during the premenstrual stage of regression[110]

Slowing of the circulation continues until a condition of *stasis is reached The more extensive the* regression the longer the duration of stasis and vice versa[112] Finally 4 24 hours before bleeding begins the basal segments of the spiral arteries become constricted This phenomenon is characteristic and constant as regards both occurrence and timing in relation to the onset of bleeding It gives rise to a gradually developing ischemia *The* constriction persists throughout the period of menstrual bleeding However following the initial ischemia *individual arteries relax temporarily and when this* happens hemorrhage occurs The endometrial basal arteries do not constrict and the circulation in the basalis continues uninterrupted throughout the menstrual period

The menstrual hemorrhage is primarily arterial resulting from rupture of the wall or an arteriole or capillary Blood escaping thus may form a hematoma which subsequently ruptures through the endometrial epithelium It may escape without forming a hematoma Diapedesis also occurs and may be repetitive It is interrupted by constriction of the *appropriate coiled artery* Venous hemorrhage accounts for about 25 per cent of the blood loss It takes place in areas of previous hemorrhage or desquamation and may be direct or reflux

Following the initial relaxation which permits the inflow of blood and rupture of the vessel the artery again constricts becoming occluded 1 24 hours after the initial hemorrhage As long as it is not completely contracted some blood may ooze from a *break in its wall Or* secondary hemorrhages may occur within 15 *minutes after fright or the intra* venous injection of epinephrine Blood escaping in secondary hemorrhage clots almost immediately but clotting is not characteristic of the other types of menstrual hemorrhage

Individual arteries relax and bleed independently In a monkey bearing more than one intra ocular *endometrial transplant different stages of regres* sion and bleeding may be seen at the same time in different transplants In fixed tissues several different stages may be seen in specimens from the same uterus[13, 94]

Desquamation begins toward the end of the first day of bleeding It takes place in areas in which bleeding has occurred and usually after some restoration of the vascular bed has been accomplished It rarely occurs during bleeding In denuded areas veins and arterioles are often found projecting into the uterine lumen The free ends are usually thrombosed but may be wide open They may persist for some time but are eventually shed[110] In areas not too markedly dehydrated whole arterial fields may be sloughed off along with the surrounding endometrial tissue[99] Many of the desquamated cells are *viable as shown by the fact that growth* of both epithelial and stromal elements has been obtained in cultures of material removed from the uterine lumen[38, 107]

The process of desquamation is usually described as a crumbling of tissue and dehydration appears to be of some importance in relation to this phenomenon *The amount of tissue lost is directly* proportional to the degree of development of the coiled arteries irrespective of the occurrence or nonoccurrence of ovulation[1 0]

Although individual hemorrhages are terminated by arterial contraction bleeding in either a localized area or in the endometrium as a whole is arrested *by re establishment of circulation in sound vessels* In the monkey repair of the vascular bed is accom

Menstruation is not the only endometrial growth limiting mechanism[89 90 97] Nor is it the only type of uterine bleeding[14] Other types of physiologic bleeding from which it should be distinguished are implantation bleeding and bleeding at the time of ovulation that is to say at the ovulatory stage of the cycle[20] These two types of bleeding differ fundamentally from menstrual bleeding in that they occur during growth phases of the cyclic activity A distinction should also be made between normal menstrual bleeding atypical menstrual bleeding and abnormal menstrual bleeding particularly with reference to the status of the vascular mechanisms involved in the production of the bleeding and the implications concerning the normality or abnormality of ovarian function Apparent normality of external bleeding does not necessarily imply normality of all the cyclic phenomena[97 135]

Little is known concerning the mechanisms which control endometrial regression Markee has discussed the role of the stromal capillaries pointing out that the decrease in intracapillary pressure which may be presumed to accompany the decrease in rate of flow late in the postovulatory phase may account for the withdrawal of part of the fluid from the stroma hence part of the regression[113] The role of the lymphatics merits investigation

Faulkner utilizing injection technics in a study of the veins of the human uterus found cyclic variations in the injectability of veins in the submucosal zone of the myometrium Good injection of this area was achieved in specimens removed during menstruation But in the postmenstrual period the veins in this zone were uninjectable and an increase in the compactness of the musculature in the area in question was observed[56 7] Sphincter like arrangements in the myometrium and interweaving of bundles of the circular layer of the myometrium with the tunica media of the larger vessels have been observed in the human uterus[9 1 3] and also in the macaque[130] These may be of significance in the control of blood flow The fact that endometrial tissue transplanted to the eye can function in an essentially normal fashion indicates that the role of the myometrial vessels can be assumed by nonspecific vascular connections But quantitative differences between transplant and endometrium *in situ* may be significant in relation to the questions of control of the endometrial circulation

Data on comparative aspects of menstrual physiology are scant but the evidence available emphasizes the relationship of menstrual bleeding to quantitative aspects of premdatory vascular growth in the endometrium According to Asdell bleeding into the uterine cavity at the beginning of regression of the corpus luteum appears to be widespread in primates but it is not sufficient in amount to produce overt menstruation except in the Old World primates The smaller amount of bleeding implies less tissue destruction in the endometrium[9] A comparison of conditions in certain New and Old World primates affords evidence on this point In the baboon[98 200] and chimpanzee[57 122 195] the nonpregnant cycle is characterized by arterial growth coiling of the arteries[88] and menstrual bleeding of macroscopic proportions Kaiser found that the arterial fields in these species were comparable to those of the macaque at comparable stages of the cycle In platyrrhine monkeys of the species Cebus the endometrial arteries are poorly developed as compared with macaque and exhibit little or no evidence of premdatory growth The endometrial desquamation is correspondingly slight and the menstrual hemorrhage is of microscopic proportions[74 98]

Of particular interest from the evolutionary point of view is the elephant shrew (*Elephantulus myurus jamesoni*)—usually classified among the Insectivora—which is a possible connecting link between primates and the sub primate species

Elephantulus exhibits a menstrual process similar in principle to that found in primates[170] The uterus in this animal is Y shaped consisting of a median part with two long horns Two young are borne per litter one in each uterine horn Implantation is restricted to a small and well circumscribed area The entire endometrium exhibits the proliferative reaction but the luteal reaction and bleeding are restricted to essentially the area in which implantation is possible In the nonpregnant cycle a polyp like body develops in this area as the corpus luteum begins to wane At the end of diestrus bleeding occurs in the polyp cellular necrosis sets in the structure enlarges and eventually ruptures discharging the necrotic mass into the uterine lumen Regeneration proceeds concurrently with menstruation is very rapid and may overlap the proliferative phase There is a direct correlation between the amount of tissue destroyed during menstruation and the size of the polyp prior to onset of the destructive process[17]

HORMONAL CONTROL OF MENSTRUATION

The cyclic changes in the endometrium are under the control of the ovarian hormones The activity characteristic of the preovulatory phase is a response to stimulation by estrogen whereas the phenomena characteristic of the postovulatory phase represent sequential stages in the total response to both estrogen and progesterone Endometrial regression which in the species under discussion includes menstruation is the terminal stage of the total response to a single cycle of stimulation by the ovarian hormones The available evidence indicates that the transition from growth to regression is precipitated normally

words the immediate cause of bleeding is circulatory deprivation which develops gradually and continues *throughout the period of bleeding* The circulatory deprivation involves only the spiral arteries and the area supplied by them The subterminal circulation is not affected and the endometrial basal arteries contribute significantly to the ultimate reorganization of the vascular bed

The initial constriction of the spiral arteries which invariably precedes the onset of flow appears to be the primary local mechanism for achieving the degree of ischemia required to assure rupture of the arteries The subsequent relaxation constriction relaxation controls the onset of bleeding and the blood loss and contributes to re establishment of the circulation in *the functionalis* The staggering of arterial activity may also be a factor in controlling the blood loss and restoring the circulatory equilibrium in the late stages of regression

Markee advanced the following hypothesis concerning possible relationships between stasis vasoconstriction and hemorrhage The excessive coiling and buckling of the arteries caused by the shrinkage of the endometrium during the premenstrual stage of regression might be responsible for the stasis The stasis in turn would cause necrosis of the endometrium and weakening of the vessel walls A substance similar to the necrosin of V Menkin[121] might emanate from the degenerating tissue and act upon an uninjured part of the artery causing the vasoconstriction Variations in the concentration of the injurious substance could account for the temporary relaxation and subsequent constriction of the individual arteries[110]

This hypothesis is based on data obtained from direct observation of menstruation in living animals and upon the demonstration that necrosin does cause constriction of the spiral arteries in intra ocular endometrial transplants[111] And the possibility that some such sequence of events is involved in the production of the degree of circulatory deprivation necessary to produce rupture of the well developed arteries of the human and macaque has not been eliminated However Bartelmez has minimized the importance of the excessive coiling of the arteries The arterial constriction which under normal conditions affects the radial arteries as well as the spirals[16 17 41] is considered sufficient to account for the stasis[16]

In any case arterial coiling is not the cause of menstruation Disproportionate arterial growth is the ultimate cause of both the coiling and the bleeding Menstruation itself results from arterial growth circulatory deprivation of extreme degree arterial damage resulting from the circulatory deprivation inflow of blood under pressure *and arterial rupture This* combination of phenomena comprises the local cause of menstrual bleeding and the sequence the quantitative interrelationships involved and the timing in relation to each other are all integral features of the mechanism by which the bleeding is produced

The fundamental consequences of menstruation are desquamation of the terminal segments of the spiral arteries and the tissues supplied by them and reorganization of the endometrial vascular bed at the functional level characteristic of the nadir of the cyclic activity The tissue desquamated was destined to provide the point of contact between the maternal circulation and the circulation of an embryo which failed to materialize Menstruation is then a vascular growth limiting mechanism reciprocal to growth promoting in the nonpregnant cycle of the human and macaque Its relative importance may be indicated by the following figures In the human the rate of endometrial growth is of the order of 3 mm per cycle Intra-ocular endometrial transplants in the monkey approximately double in thickness in each of the two subdivisions of the growth phase While it is impossible to determine accurately the length of the coiled arteries in living transplants it is possible to estimate changes in the length of a single artery during a single cycle The ratio between the length of a given artery and the thickness of the endometrium may increase from 2 1 at the beginning of the growth phase to 5 1 at the end of the primary period of growth and to 10 1 at the end of the secondary period of growth[113]

Endometrial regression restores the basal blood volume vascular channel volume hydration volume endometrial tissue volume and the topographical relationships between endometrial tissue and vascular bed characteristic of the basal level of functional activity Menstruation restores the basal vascular channel volume and basal vascular pattern and contributes to reduction in the local blood volume and restoration of the equilibrium between blood volume and channel volume

Menstruation as it occurs in the human being and in the rhesus monkey has been defined as a specific vascular growth limiting mechanism reciprocal to and acting in sequence with a specific vascular growth promoting mechanism in species in which marked prenidatory arterial growth is required to assure delivery of the nidatory blood volume requirement to the place of nidation at the time of nidation[138] Acting in conjunction with the circulatory mechanisms which control endometrial regression the mechanism contributes to maintenance of such fundamental aspects of the cyclic activity as (1) synchronization of successive *cycles of endometrial* vascular development with successive cycles of ovum development and (2) the spatial relationship be*tween maternal* and fetal circulatory axes characteristic of the species

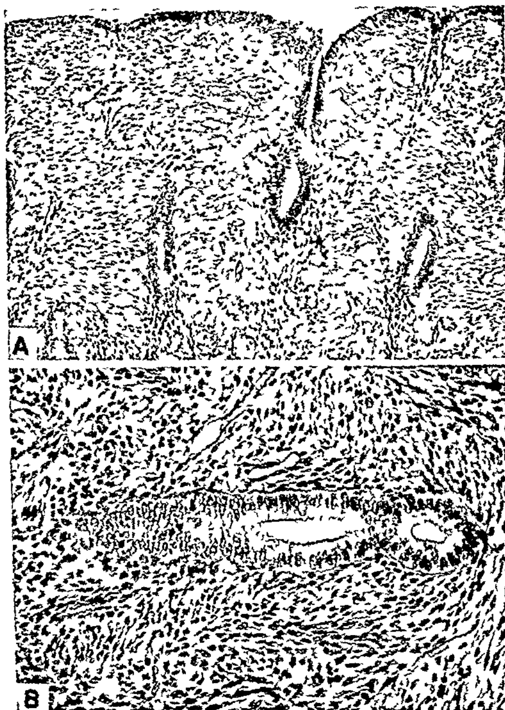


PLATE 4

Fig 13 Early proliferative endometrium *A* Surface epithelium thin glands sparse narrow and straight (× 150) *B* Few mitoses in glands and stroma little pseudostratification of gland nuclei (× 400) (Reproduced courtesy of Noyes, Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 6 1950 fig 2)

by a decline in the level of ovarian activity The original basis for this concept was the observation that any one of a number of procedures involving hormone withdrawal—for example cessation of treatment with one or both ovarian hormones in an oophorectomized female—would precipitate bleeding similar to normal menstruation in amount duration and local vascular phenomena [5, 110, 138] The chronological relationship of menstruation to onset of regression in the corpus luteum [1, 2] indicates that a reduction in the level of stimulation does normally precede the onset of menstruation and this is also indicated by the data on cyclic fluctuations in the amount of estrogen and progesterone demonstrable in the blood and urine [25, 61, 8, 115]

The blood estrogen level which is minimal during the menstrual period rises shortly before ovulation and begins to decline about a week before the onset of bleeding Markee and Berg noted a drop from about 60 I U per liter to about 30 I U per liter beginning about 6 days before the onset of menstruation [115] Forbes [61] using the Hooker Forbes technic for assay of progesterone in the blood [92] reported a rise in progesterone during the luteal phase followed by a decline In most cases however progesterone did not disappear until after the onset of the menses Using the same technic Forbes Hooker and Pfeiffer [6] and Bryans [28] were able to demonstrate a decrease in progesterone preceding menstruation in rhesus monkeys

In a recent review Browne Venning and Henry have summarized their extensive observations on correlations between excretion of sodium pregnanediol glucuronidate and the onset of menstrual bleeding [25] They found that in cycles varying from 23 to 42 days in length the average time from onset of pregnanediol excretion to onset of the following menstrual period was 12 13 days with the interval between end of pregnanediol excretion and onset of menstruation varying from 1 to 4 days rarely longer Except in certain types of abnormality these authors had not found pregnanediol present for more than a few days without evidence of progestational action on the endometrium However they had obtained clinical evidence that amounts of progesterone inadequate to produce progestational changes may determine onset of bleeding [25] Similar findings in the monkey have been reported by Hisaw [88]

Klopper has recently reported a study of pregnanediol excretion in which he used a technic capable of measuring the small amount of pregnanediol originating from the adrenal as well as the larger contribution of the ovary He found that menstrual bleeding did not start until urinary pregnanediol levels had fallen to 2 5 mgm or lower Pregnanediol output continued to decline during the first few days of bleeding and remained at a basal level of approximately 1 mgm per 24 hours during the proliferative phase An increased excretion began some 2-3 days before ovulation as determined by basal temperature During the luteal phase the amounts excreted varied from 2 to 7 mgm per 24 hours the variations being related in part to age and parity [105]

The case then for normal ovulatory menstruation seems fairly clear cut But anovulatory menstruation anomalies and frank abnormalities of the menstrual function are another matter It is well known that bleeding may occur during treatment with a constant daily dosage of estrogen [67, 108] Phelps has shown that bleeding may occur in the monkey in any given relationship to the level of hormonal stimulation and fluctuations in same [135] And there is abundant clinical and experimental evidence that bleeding in the human female may take place in almost any given combination of hormonal circumstances So there is no dearth of problems

CYCLIC HISTOLOGIC CHANGES IN THE ENDOMETRIUM

As mentioned previously the growth phase of the menstrual cycle is divided into two sub phases synchronous respectively with the preovulatory and postovulatory phases of the ovarian cycle The preovulatory phase is characterized by an increase in the level of circulatory activity imbibition of water and proliferation of the endometrial cells The postovulatory phase is characterized by maturation of the spiral arteries development of the vascular bed in the nidatory area and secretion of nutritive materials into the uterine lumen

These changes are evidenced by the well known histologic changes demonstrable in fixed sections Rock and Hertig and their co workers have studied these changes exhaustively in connection with their studies on early human embryos [87, 14, 148] They have worked out morphologic criteria which indicate the relative duration of progesterone action and provide a basis for seriation of endometrial specimens according to the relative duration of the response to progesterone For practical purposes they have assigned dates to sequential periods which can be distinguished on the basis of the criteria referred to The dates applied are as of the days of a classic 28 day cycle assuming that ovulation occurs on the 14th day and menstruation on the 28th day There is some variation in the duration of the secretory phase hence the dates are arbitrary and represent sequential phases characterized by the histologic appearance peculiar to each rather than actual days of the cycle The numerical designation indicates therefore a certain morphologic picture and also the position of that picture in the sequence of changes which com

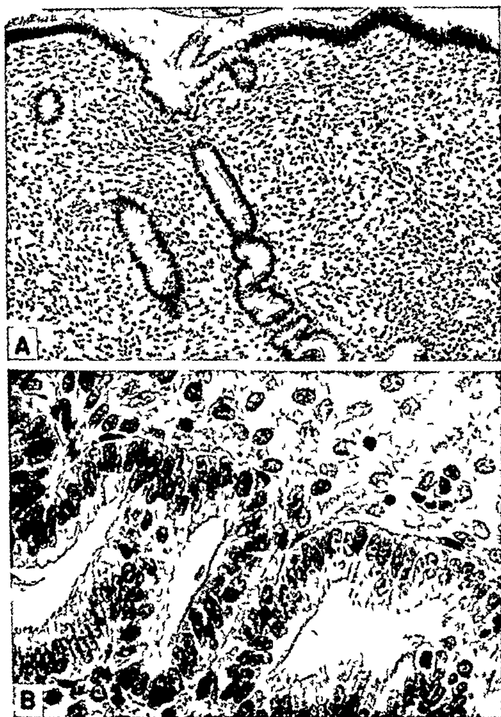


PLATE 6

Fig 15 Late proliferative endometrium *A* Glands tortuous stroma usually quite dense (× 150) *B* Epithelial nuclei are pseudostratified and are oval in shape (× 400) Compare with Figure 3 (Plate 1) and with late proliferative stage Plate 16 (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 9 1950 fig 4)



PLATE 5

Fig 14 Endometrial specimen showing an intermediate degree of proliferation A Glands slightly tortuous surface epithelium tall columnar Extracellular fluid is not always as marked as in this section (X 150) B The glands show numerous mitoses with pseudostratification becoming marked Noteworthy is the naked nucleus type of stromal cell with fine anastomosing processes (X 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 8 1950 fig 3)

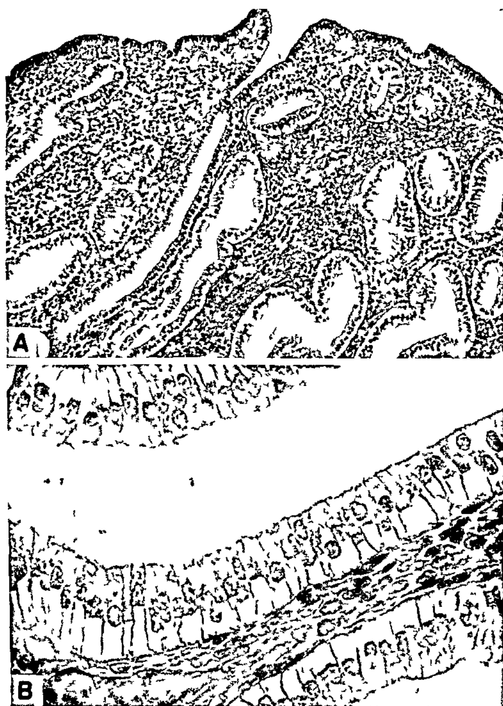


PLATE 8

FIG 17 Endometrium representative of third postovulatory day *A* Gland nuclei are pushed to the center of the epithelial cells with cytoplasm above the vacuoles below them (× 150) *B* Gland mitoses rare pseudostratification decreasing (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 12 1950 fig 7)

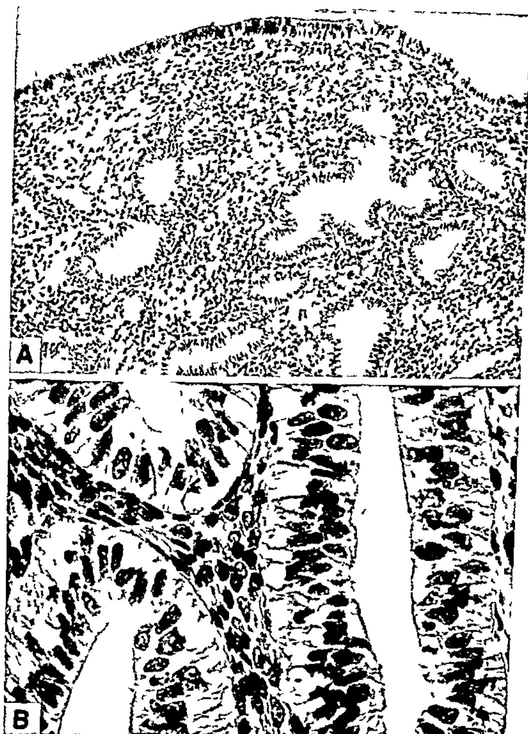


PLATE 7

Fig 16 Endometrium representative of second postovulatory day *A* Glands tortuous stroma dense cells consisting of nearly naked nuclei (× 150) *B* Gland mitoses very numerous pseudostratification of nuclei exaggerated by subnuclear vacuoles (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 10 1950 fig 5)



PLATE 8

FIG. 17 Endometrium representative of third postovulatory day *A* Gland nuclei are pushed to the center of the epithelial cells with cytoplasm above the vacuoles below them. (× 150) *B* Gland mitoses rare pseudostratification decreasing (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 12 1950 fig 7)



PLATE 9

FIG 18 Endometrium representative of fourth postovulatory day A Gland nuclei are returning to the base of the cells Wisps of secretory material are present in the lumina (X 150) B Some vacuoles are pushed past the nucleus on their way to empty glycogen into the lumen Mitoses and pseudostratification of nuclei absent (X 400) This is the stage of arrival of the ovum in the uterus Compare with Figure 4 (Plate 1) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 13 1950 fig 8)

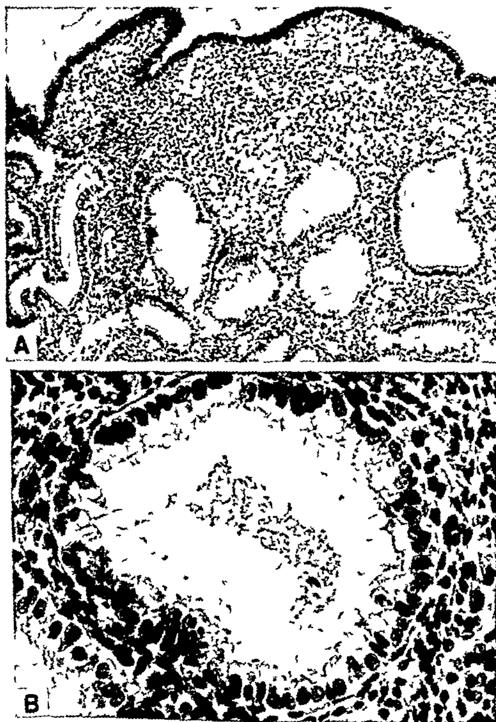


PLATE 10

Fig 19 Endometrium representative of sixth postovulatory day corresponding to beginning implantation in the fertile cycle A Secretion in the gland lumen, at its peak. Beginning of accumulation of extravascular fluid in the stroma (× 150) B Subnuclear vacuoles, rare nuclei, round and basally located Secretion prominent in gland lumen. (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 15 1950 fig 9)

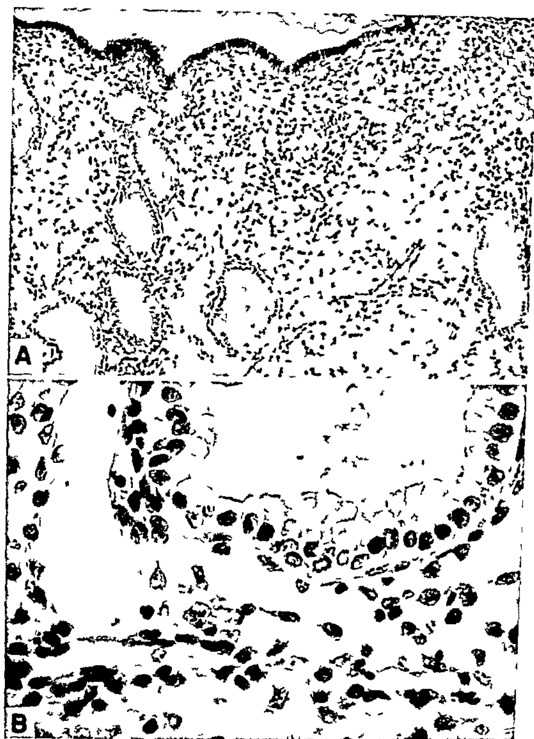


PLATE 11

Fig 20 *Endometrium representative of the eighth postovulatory day A Extracellular fluid maximal* The walls of the spiral arterioles are not prominent (× 150) *B* The stromal cells still appear as small dense naked nuclei widely separated by extracellular fluid Glandular secretion still active but subsiding (× 400) (Reproduced courtesy of Noyes Hertig and Rock, Dating the endometrial biopsy Fertility and Sterility 1 16 1950 fig 10)



PLATE 12

FIG 21 *Endometrium representative of the ninth postovulatory day A Spiral arterioles become prominent due to condensation of their surrounding stroma* (× 150) *B* Both the nuclei and the cytoplasm of the periarteriolar stromal cells are enlarging This is the earliest predecidual reaction (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 1" 1950 fig 11)

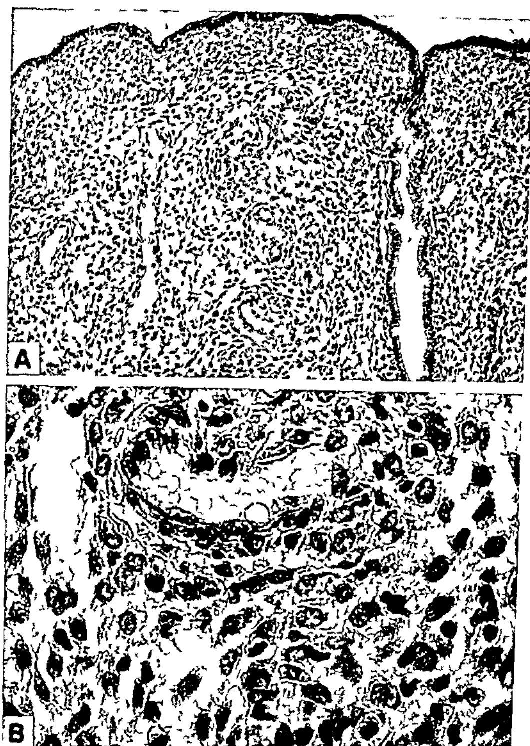


PLATE 13

FIG 22 Endometrium representative of the tenth postovulatory day A Spiral arterioles and surrounding predecidua still more prominent and extracellular fluid subsiding (× 150) B Thickening of the periarteriolar predecidual cuff Stromal mitosis evident (× 400) (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 18 1950 fig 12)

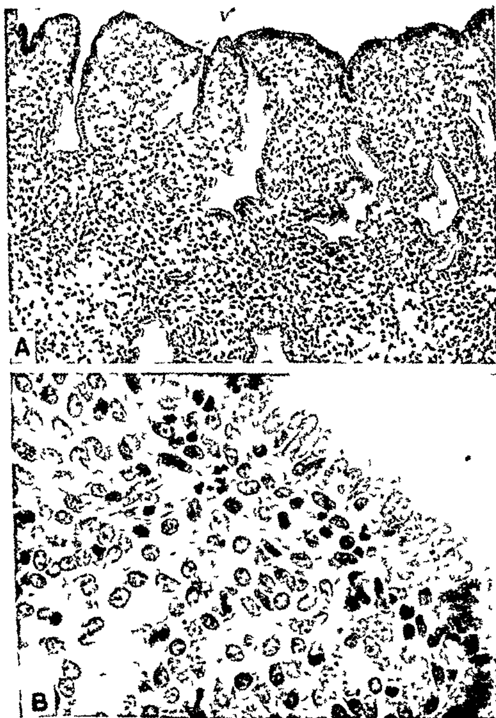


PLATE 14

Fig 23 Endometrium representative of the eleventh postovulatory day *A* Pseudodecidua begins to differentiate under the surface epithelium Stroma of the stratum spongiosum still contains extracellular fluid except in areas near a spiral arteriole (× 150) *B* Round cell infiltration accompanies predecidual differentiation Stromal cells swell to become predecidual in type (× 400) Compare with progestational and early pregnancy stages illustrated in Plate 16 In the nonpregnant cycle the transition from growth to regression takes place in the endometrium at about this time (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 19 1950 fig 13)

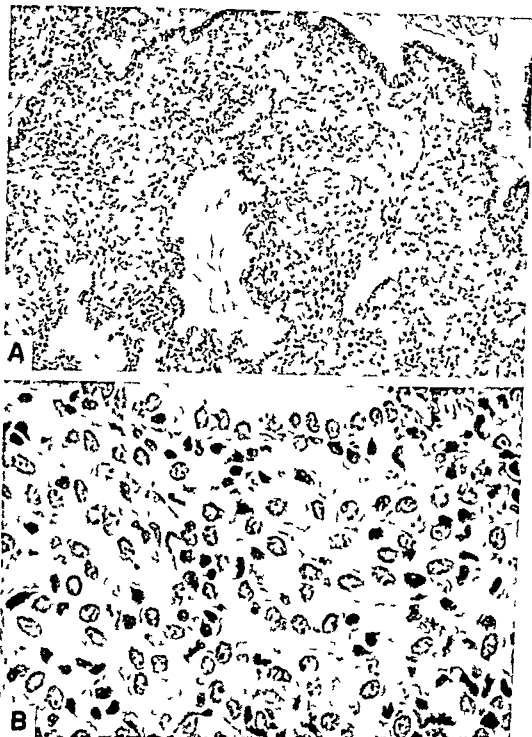


PLATE 15

Fig 24 Endometrium representative of the thirteenth postovulatory day of an ideal 28 day cycle i e end of the nonbleeding phase of the menstrual cycle *A* Intraluminal secretion inspissated Glandular epithelium secretorily exhausted (× 150) *B* Polymorphonuclear and other leukocytic infiltration of the stroma which shows marked predecidual changes (Reproduced courtesy of Noyes Hertig and Rock Dating the endometrial biopsy Fertility and Sterility 1 20 1950 fig 14)

In evaluating endometrial specimens obtained late in the intermenstrual interval it should be borne in mind that the menstrual cycle is not synchronous with the growth regression cycle On the final day of the nonbleeding phase of an actual menstrual cycle the endometrium would be in the premenstrual stage of the phase of regression Cf Figure 6 (Plate 2) *Generally recognized criteria for differentiating early stages of endometrial regression from late* stages of endometrial growth have not been worked out, but such criteria would be of great value in the study of *endometrium*

prise the response of the endometrium to a single cycle of stimulation by the ovarian hormones.[1, 5]

Although this method has its limitations for both clinical and investigative work, it has proved very useful for obtaining information concerning various aspects of ovarian function.

Figures 13-24 are photomicrographs of endometrium seriated on the basis of the criteria referred to. They illustrate the types of functional activity and histologic criteria characteristic of the preovulatory and *postovulatory phases of the cycle and sequential* stages in the postovulatory phase and in the total endometrial response to a single cycle of stimulation by the ovarian hormones.

METABOLISM

The cyclic metabolic and enzymatic activity in the endometrium can be correlated with the changes in vascular activity, the growth and secretory activity of the endometrial cells, the normal changes in qualitative and quantitative aspects of the hormonal stimulus, and with the presumed requirements of the embryo (real or potential) as it progresses from the ovary to its attachment in the uterus. The metabolic activity reaches its peak at about the time of arrival of the ovum in the uterus and remains high until after the transition from growth to regression. Thereafter there is a decline, although certain types of activity remain high during the phase of regression.

Histochemical preparations reveal that most of the biochemical alterations are due to changes in the glandular and surface epithelium, whereas the stroma and blood vessels contribute only slightly. McKay, Hertig, Bardawil and Velardo compared histochemical observations with chemical measurements of their own and of other workers and found that semiquantitative estimates based on histochemical observations were in essential agreement with the chemical measurements.[115] Cyclic variations in metabolic activity demonstrated by these investigators are illustrated in Plates 16 and 17.

Following ovulation the human ovum remains in the tube for about three days, and then lies free in the uterine cavity for about three more days while in the late morula and blastocyst stages. On the 5-6th developmental day it begins its interstitial implantation. While it is free in the uterine cavity the only exogenous source of nutrition for the blastocyst is the secretion of the surface and glandular epithelium. During this period the endometrial secretions contain small amounts of ribonucleoprotein, nonspecific esterase, and 5-nucleotidase, *and relatively* large amounts of glycogen, mucus (mucopolysaccharides, glycoprotein), and alkaline phosphatase.[11]

At the stage of beginning implantation the surface epithelium contains abundant glycogen,[75] acid phosphatase activity,[70, 71, 115] dehydrogenase activity,[90] and lipid material.[5] The endometrium is at its metabolic peak with respect to oxygen uptake,[78] aerobic glycolysis, succinic and malic dehydrogenase activity, cytochrome activity, and adenosinetriphosphatase activity.[167]

In the proliferative and progravid phases a relation seems to exist between the enzymatic activity and functional activity such as has been shown for other tissues.[8] The proliferative phase is characterized by high levels of *ribonucleoprotein*[1, 4] and alkaline phosphatase activity[11, 1, 5] and by small amounts of acid phosphatase activity, glycogen, and glycoprotein. As progesterone becomes dominant following ovulation, glycogen, which is sparse in the preovulatory phase, becomes abundant.[8, 93, 134, 1, 5] This phase is also characterized by large amounts of acid phosphatase activity and glycoprotein and small amounts of ribonucleoprotein and alkaline phosphatase activity.[1] The association of ribonucleoprotein and protein synthesis is well known. Caspersson has shown a close relationship between rapid growth of tissues and an increased content of ribonucleoprotein.[3] There is a close correlation between the quantitative changes in ribonucleoprotein[103] and the mitotic activity in the functionalis, which reaches its peak at the stage of *ovulation* and drops off sharply to virtually zero at the stage of nidation.[19, 4, 1, 0]

Stuermer and Stein[167] and Hagerman and Villee[7] demonstrated slight peaks of approximately equal height in the oxygen uptake of midproliferative and midprogestational endometrium. In general the rate of metabolism of proliferative and progestational endometrium was quite similar. It appears from the observations of these investigators that maturation of the endometrium consists in part of conversion of cellular energy from production and utilization of protein to the production and utilization of carbohydrate substances and acid phosphatase. Mucopolysaccharides[117] and fat[83] may be added to the latter group. Stuermer and Stein found that endometrial maturation is also characterized in part by a shift from predominantly anaerobic glycolysis during the growth phase to predominantly aerobic glycolysis during the secretory phase.[16]

Ribonucleoprotein increases to a maximum late in the proliferative phase and decreases during the progestational phase.[16] Alkaline phosphatase activity also increases during the proliferative phase, reaching a peak about the time of ovulation, and decreasing during the secretory phase.[8, 11, 1, 2, 7, 98, 118] It is abundant in the endothelial cells of the spiral and myometrial arterioles throughout the growth phase.[11]

Glycogen is present in small amounts during the proliferative phase, but increases under the influence of progesterone and reaches a peak at about the time of arrival of the ovum in the uterus. It decreases

in the final quarter of the cycle [72] Jones Wade and Goldberg demonstrated a reciprocal relationship between adenosinetriphosphatase and glycogen i e when glycogen was high ATP was low and vice versa And this was found in different areas of the same specimen as well as in different specimens *Moreover although the phosphate release was highest at the peak of the progestational phase and paralleled the serum progesterone and pregnanediol curves it was inhibited by progesterone in vitro* [95]

Acid phosphatase activity is low during the proliferative phase and is present in increasingly larger amounts as the progestational phase progresses McKay Hertig Bardawil and Velardo found it in greatest abundance in menstrual endometrium [118] Page Glendening and Parkinson found a rise in the activity of an endometrial proteolytic enzyme beginning late in the proliferative phase and reaching a peak at the time of menstruation [13]

Implications of the quantitative changes in metabolic activity concerning the relationship of these phenomena to the action of the ovarian hormones have been confirmed in animal experiments [145 146 169] To mention only a few observations Telfer demonstrated that the injection of estrogen into oophorectomized rats increased the production of ribonucleoprotein by the rat uterus [171] Atkinson and Engle found abundant alkaline phosphatase activity in the endometria of estrogen treated oophorectomized monkeys [1] Hagerman and Villee have demonstrated an increase in the endometrial metabolic rate in response to estrogen both *in vitro* and *in vivo* [73] Fishman and his co workers have shown that endometrial beta glucuronidase activity is increased under the influence of estrogen [59 125]

DURATION OF THE MENSTRUAL CYCLE AND TIME OF OVULATION

In recent years a wide variety of technics has been developed for estimating the time of ovulation in the menstrual cycle and for obtaining information about other aspects of ovarian function as well Except for direct observation of ovulation [47] or recovery of ova [6] or blastocysts [83 147 148] from the genital tract these procedures involve use of indirect indicators of ovulation Hence each has certain disadvantages and sources of error On the other hand each has proved to be of some practical clinical value [165] The technics referred to include endometrial biopsy [18 27 9 101 125 174] variations in basal body temperature known to be correlated with ovarian activity [8 153 160 17 3 184 1 7] changes in viscosity and other properties of the cervical mucus [1 32 15 183 203] vaginal smears [43 4 133] bioelectric correlates [72] measurements of progesterone [61] and pregnanediol in blood and urine [58 149 186] histology of the corpus luteum [17 24 29 3 10] and the test developed by Farris which depends on variations in pituitary gonadotropin excretion [37 53 5]

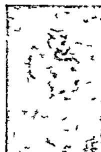
Of interest in the present connection are data accumulated with these technics concerning the chronologic relationships between menstruation and ovulation in the human female

Data on duration of the menstrual cycle in the human being and in the macaque indicate a modal value for each species of about 28 days with a range of variation of about 7 days [14 33 101] Under strictly normal conditions the range in women is probably less than this In a study of variations in the duration of successive cycles in a group of 480 women Miller found a variation of 1 9 days with a range of variation of 6 days or less in 85 per cent of the cases [1??] From this and further studies of the same type Miller concluded that the menstrual cycle of a normal woman varies continually between a certain maximum and a certain minimum number of days and that the cycle of the average healthy woman varies between a minimum of 26 27 days and a maximum of 30 32 days [1 2]

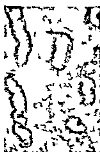
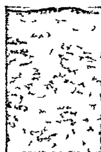
Data from the sources referred to above are in reasonable agreement in indicating that ovulation normally occurs within a range of about 14 ± 6 days after the onset of the preceding menstrual period with highest frequencies days 12 15 mode at day 13 The postovulatory phase is less variable than the preovulatory in women [13 33 151 172] Hartman s data on the macaque indicate a similar range of variation with highest frequencies days 11 13 mode day 13 Data on duration of the interval between ovulation and onset of the subsequent menstrual period show a range of variation of 7 23 days [76 78] Corner s findings indicate less variation in the duration of corpus luteum function From histologic studies he concluded that the average duration of the luteal phase in the rhesus monkey is 15 days [35]

It is self evident that the quantitative aspects of the menstrual cycle which are subject to variation must over a period of x cycles vary in reciprocal directions Otherwise the activity would not be cyclic Moreover the single menstrual cycle is not a unit unto itself—a chemical reaction in a test tube or an arm from which all the muscles except the biceps have been removed Its quantitative aspects are profoundly influenced by the quantitative aspects of stimulus response cycles that have gone before It is also influenced by the subsequent cycle which of course has already started in pituitary and ovaries before it becomes manifest in the endometrium The influence of previous cycles is mediated in part by changes induced in the endometrial vascular bed These may be limited to the current cycle or they may be carried over into future cycles The specific vascular morphology at the beginning of any given

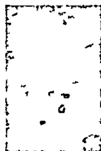
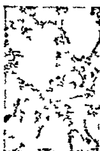
R b n cl op otein

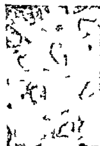

Glycogen


Alk l e
Ph photas

Ac d
Ph ph t



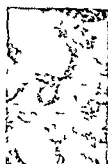
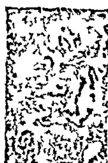
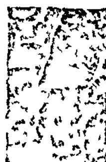


Late P olf t e R 50	25-d y P gest t l R 63	E ly P g cy R 109

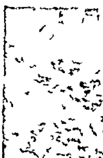
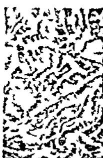
PLATE 16

Histochemical reactions characteristic of the p oliferative progestational and early pregnancy stages of the normal cyclic acti ity in the human end ometrium The three types of normal endometrium are arranged vertically and the histochemical reactions h rizontally for comparison of the se eral reactions in different stages of development (Reproduced courte y of McKay Hertig Bardawil and Velardo Histochemical observations on the endometrium I Normal endometrium Obstetrics & Gynecology 8 2) 1956 plate I)

Ribonucleoprotein

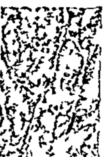




Glycogen

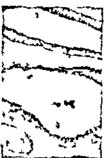




Alkaline Phosphatase





Acid Phosphatase





Cystic Hyperplasia R 82 R 105	Carcinoma in situ R 57	Adenocarcinoma R 112

PLATE 17

Histochemical comparison of three pathologic conditions in endometrium cystic hyperplasia carcinoma *in situ* and adenocarcinoma The three pathologic conditions are arranged vertically and the several histochemical reactions horizontally (Reproduced courtesy of McKay Hertig Bardawil and Velardo Histochemical observations on the endometrium II Abnormal endometrium Obstetrics & Gynecology 8 149 1956 plate I)

cycle has a significant influence upon the quantitative aspects of that cycle and in particular upon the quantitative aspects of menstrual bleeding[135]

In a word the maternal test tube *enters* into the reaction Every day it is a different test tube

BIBLIOGRAPHY

1 Abarbanel A R 1948 Artificial reproduction of the cyclic changes in cervical mucus in human castrates West J Surg 56 26-34

2 Allen E 1927 Menstrual cycle of monkey Macacus rhesus observations on normal animal effects of removal of ovaries and effects of injections of ovarian and placental extracts into spayed animals Contrib Embryol 19 1-44

3 —— and Doisy E A 1923 An ovarian hormone preliminary report on its localization extraction and purification and action in test animals J A M A 81 819 21

4 Allen E Danforth C H and Doisy E A 1939 Sex and Internal Secretions Baltimore Williams & Wilkins ed 2 pp 452 629 and 931 85

5 Allen E Hisaw F L and Gardner W U 1939 Endocrine functions of ovaries in Allen E Danforth C H and Doisy E A Sex and Internal Secretions Baltimore Williams & Wilkins ed 2 pp 452-629

6 Allen E Pratt J P Newell Q U and Bland L J 1930 Human tubal ova related early corpora lutea and uterine tubes Contrib Embryol 22 45-75

7 Allen W M 1930 Physiology of corpus luteum V Preparation and some chemical properties of progestin hormone of corpus luteum which produces progestational proliferation Am J Physiol 92 174 88

8 Arzac J P and Blanchet E 1948 Alkaline phosphatase and glycogen in human endometrium J Clin Endocrinol 8 315-24

9 Asdell S A 1946 Patterns of Mammalian Reproduction Ithaca N Y Comstock pp 96-132

10 Astwood E B 1939 Changes in weight and water content of uterus of normal albino rat Am J Physiol 126 162-70

11 Atkinson W B 1950 Studies on the Effects of Steroid Sex Hormones on Alkaline Phosphatase in the Endometrium in Engle E T Menstruation and Its Disorders Springfield Ill Thomas pp 3-21

12 Atkinson W B and Engle E T 1947 Studies on endometrial alkaline phosphatase during the human menstrual cycle and in the hormone treated monkey Endocrinology 40 327-33

13 Bartelmez G W 1933 Histological studies on menstruating mucous membrane of human uterus Contrib Embryol 24 141 86

14 —— 1937 Menstruation Physiol Rev 17 28-72

15 —— 1953 Factors in the variability of the menstrual cycle Anat Rec 115 101 20

16 —— 1956 Premenstrual and menstrual ischemia and the myth of endometrial arteriovenous anastomoses Am J Anat 98 69 84

17 Bartelmez G W with the collaboration of Corner G W and Hartman C G 1951 Cyclic changes in the endometrium of the rhesus monkey (Macaca mulatta) Contrib Embryol 34 99 144

18 Bergman P 1950 Sexual cycle time of ovulation and time of optimal fertility in women Studies on basal body temperature endometrium and cervical mucus Acta obst et gynec Scandinav 29 suppl 4 1 122

19 Bensley C M 1951 Cyclic fluctuations in the rate of epithelial mitosis in the endometrium of the rhesus monkey Contrib Embryol 34 89 98

20 Bromberg Y M and Bercovici B 1956 Mechanism of ovulation bleeding Acta endocrinol 23 33-8

21 Boving B G 1952 Rabbit trophoblast invades uterine epithelium overlying blood vessels Anat Rec 112 314

22 —— 1955 Uterine epithelial cells guide trophoblast hemotropism Anat Rec 121 426

23 Brewer J I 1942 Studies of human corpus luteum evidence for early onset of regression of corpus luteum of menstruation Am J Obst & Gynec 44 1048-62

24 Brewer J I and Jones H O 1947 Time of ovulation Am J Obst & Gynec 53 637-44

25 Browne J S L Venning E H and Henry J S 1950 Corpus Luteum Hormone in Gynecology in Meigs J V and Sturgis S H Progress in Gynecology New York Grune vol II pp 239 52

26 Bryans F E 1951 Progesterone of blood in menstrual cycle of monkey Endocrinology 48 733-40

27 Burch J C and Phelps D 1950 Endometrial Biopsy in Meigs J V and Sturgis S H Progress in Gynecology New York Grune vol II pp 110-18

28 Burton J F 1953 The histological demonstration of alkaline phosphatase activity in human endometrium Brit J Exper Path 34 159-63

29 Buxton C L and Engle E T 1950 Time of ovulation—a correlation between basal temperature the appearance of the endometrium and the appearance of the ovary Am J Obst & Gynec 60 539 51

30 Caspersson T 1947 The relations between nucleic acid and protein synthesis Symp Soc Exper Biol & Med 1 127 51

31 Cleveland R Phelps D and Burch J C 1941 Experimental production of glandular cystic hyperplasia in castrated macaques Endocrinology 28 859 63

32 Cohen M R Stein I F and Kaye B M 1952 Spinnbarkeit A characteristic of cervical mucus Significance at ovulation time Fertil & Steril 3 201 9

33 Collett M E Wertenberger G E and Fiske V M 1954 The effect of age upon the pattern of the menstrual cycle Fertil & Steril 5 437-48

34 Corner G W 1923 Ovulation and menstruation in Macacus rhesus Contrib Embryol 15 73 101

35 —— 1945 Development organization and breakdown of corpus luteum in rhesus monkey Contrib Embryol 31 117-46

36 Corner G W and Allen W M 1929 Physiology of corpus luteum II Production of special uterine reaction (progestational proliferation) by extracts of corpus luteum Am J Physiol 88 326-39

37 Corner G W Sr Farris E J and Corner G W Jr 1950 Dating of ovulation and other ovarian crises by histological examination in comparison with Farris test Am J Obst & Gynec 59 514 28

38 Cron R S and Gey G 1927 Viability of cast-off menstrual endometrium Am J Obst & Gynec 13 645-7

39 Dalgaard J B 1946 Blood vessels of human endometrium A histological study by means of injection

and blood corpuscle colouration Acta obst et gynec Scandinav 26 342 78
40 Dalton J C 1851 Prize essay on the corpus luteum of menstruation and pregnancy Tr A M A 4 549 644
41 Daron G H 1936 The arterial pattern of the tunica mucosa of the uterus in Macacus rhesus Am J Anat 58 349-419
42 ——— 1937 Veins of endometrium (Macacus rhesus) as source of menstrual blood Anat Rec 67 13
43 De Allende I L C 1956 *Anovulatory cycle in women* Am J Anat 98 293-305
44 De Allende I L C and Orias O 1950 Cytology of the Human Vagina New York Hoeber
45 DeFeo V J and Reynolds S R M 1956 Modification of the menstrual cycle in rhesus monkeys by Reserpine Science 124 726 7
46 Dempsey E W and Wislocki G B 1946 Histochemical contributions to Physiology Physiol Rev 26 1 27
47 Doyle J B 1954 Ovulation and effects of selective uterotubal denervation Direct observations by culdotomy Fertil & Steril 5 105-30
48 Dublin W B 1939 Mitosis in normal endometrium *Proc Staff Mayo Clinic 14 783*
49 Durante G 1933 Le glomus uterin regulateur de la circulation uterine Presse med 41 2064 8
50 Eckstein P 1950 Induction of progesterone withdrawal bleeding in spayed rhesus monkeys J En *docrinol 6 405 11*
51 Eckstein P Krohn L Zuckerman S and Healy M J R 1952 The comparative potency of natural and synthetic oestrogens in the rhesus monkey J Endocrinol 8 291 307
52 Elder J H and Yerkes R M 1936 The sexual cycles of the chimpanzee Anat Rec 67 119 43
53 Farris E J 1953 Characteristics of human ovulation as determined by the rat hyperemia test Anat Rec 115 304 5
54 ——— 1948 Prediction of day of human ovulation by rat test as confirmed by 50 conceptions Am J Obst & Gynec 56 347 52
55 ——— 1946 The time of ovulation in the monkey *Anat Rec 95 337 46*
56 Faulkner R L 1945 Injection study of uterine blood vessels Am J Obst & Gynec 49 1 9
57 ——— 1951 An injection study of the blood vessels of the bleeding uterus Am J Obst & Gynec 61 766-73
58 Fischer R H McColgan S P and Chaney A L 1952 Progesterone metabolism *I Pregnanediol excretion* in the menstrual cycle Am J Obst & Gynec 63 613 19
59 Fishman W H Kasdon S C Bonner C D Fishman L W and Homburger F 1951 Beta *glucuronidase studies in women* V The menstrual cycle And serum Beta glucuronidase activity in estrogen treated postmenopausal subjects J Clin Endocrin 11 1425-35
60 Foraker A G Celi P A and Denham S W 1954 Dehydrogenase activity in normal and hyperplastic endometrium Cancer 7 100 105
61 Forbes T R 1950 Systemic plasma progesterone levels during the human menstrual cycle Am J Obst & Gynec 60 180 86
62 Forbes T R Hooker C W and Pfeiffer C A 1950 Plasma progesterone levels and menstrual cycle of monkey Proc Soc Exper Biol & Med 73 177 9
63 Gillman J 1941 The lipines in the human endometrium during the menstrual cycle and pregnancy and their relations to the metabolism of oestrogen and progestogen S Afr J Med Sci 6 59 81
64 Gilman J Personal communication to the author
65 ——— 1941 Effect of progesterone on normal human menstrual cycle J Clin Endocrinol 1 331-8
66 Gillman J and Gilbert C 1946 The reproductive cycle of the Chacma baboon (Papio ursinus) with special reference to the problems of menstrual irregularities as assessed by the behavior of the sex skin *S Afr J Med Sci 11(suppl) 1 54*
67 Gillman J 1942 Quantitative study of bleeding threshold and optimum estrogen requirements in baboon (Papio porcarius) Endocrinology 31 172-8
68 ——— 1940 Experimental studies on menstrual cycle of baboon (Papio porcarius) effect of progesterone upon first part of cycle in normal female baboons Endocrinology 26 80-87
69 Gilbert C and Gillman J 1956 The role of the adrenal and thyroid in promoting bleeding in hypophysectomized baboons (Papio ursinus) Endocrinology 58 753 66
70 Goldberg B and Jones H W Jr 1956 Acid phosphatase of the endometrium Histochemical demonstration in various normal and pathologic conditions Obst & Gynec 7 542 6
71 Goldberg B and Jones H W Jr 1953 Acid phosphatase in human female genital tract A histochemical and biochemical study Proc Soc Exper *Biol & Med 83 45-50*
72 Gott A L and Burr H S 1953 Electrical correlates of ovulation in the rhesus monkey Yale J Biol & Med 25 408 17
73 Hagerman D D and Villee C A 1953 Effects of *the menstrual cycle on the metabolism of the* human endometrium Endocrinology 53 667 73
74 Hamblett G W D 1939 Reproduction in American monkeys I Estrous cycle ovulation and menstruation in Cebus Anat Rec 73 171-87
75 Hall J E 1950 Alkaline phosphatase in human endometrium Am J Obst & Gynec 60 212 16
76 Hartman C C 1932 Studies in reproduction of monkey Macacus (Pithecus) rhesus with special reference to menstruation and pregnancy *Contrib* Embryol 23 1 161
77 Hartman C G and Speert H 1941 *Action of* progesterone on genital organs of unprimed rhesus monkey Endocrinology 29 639 48
78 Hartman C G 1944 Recovery of primate eggs and embryos methods and data on time of ovulation West *J Surg 52 41 61*
79 ——— 1944 Regeneration of monkey uterus after surgical removal of endometrium and accidental endometriosis West J Surg 52 87 102
80 Hasner E 1946 Endometriets Vasculare Cyklus Thesis Copenhagen Des Berlingske Bogtrykkeri
81 Hechter O Krohn L and Harris J 1941 The effect of estrogen on the permeability of the uterine capillaries Endocrinology 29 386 92
82 Heckel G P and Tobin C E 1956 Arteriovenous shunts in the myometrium Am J Obst & Gynec 71 199 205
83 Hertig A T and Rock J 1944 On development of early human ovum with special reference to trophoblast of previllous stage a description of 7 normal and 5 pathologic human ova Am J Obst & Gynec *47 149 84*
84 Heuser C H and Streeter G L 1941 Development of the macaque embryo Contrib Embryol 29 15-55

85 Hillebrand, H 1951 Die Bedeutung der Morgen temperaturkurven zur Bestimmung des Ovulation stermins fur den Fachgynakologen und fur den praktischen Arzt Munch med Wchnschr 93 735-8
86 Hirsch Edwin F and Martin Mary 1943 The distribution of the nerves in the adult human myometrium Surg Gynec & Obst 76 697 702
87 Hisaw F L and Astwood E B 1942 Physiology of reproduction Ann Rev Physiol 4 503 60
88 Hisaw F L 1942 Interaction of ovarian hormones in experimental menstruation Endocrinology 30 301 8
89 —— 1948 Endomentrial growth in monkeys Science 107 457-8
90 —— 1950 Factors influencing endometrial growth in monkeys (Macaca mulatta) in A Symposium on Steroid Hormones ed by E S Gordon The University of Wisconsin Press p 259
91 Hitschmann F and Adler I 1908 Der Bau der Uterusschleimhaut des geschlechtsreifen Weibes mit besonderer Beruksichtigung der Menstruation Monatschr f Geburtsh u Gynak 27 1 82
92 Hooker C W and Forbes T R 1947 Bio assay for minute amounts of progesterone Endocrinology 41 158-69
93 Hughes E C 1945 Relationship of glycogen to problems of sterility and ovular life Am J Obst & Gynec 49 10 18
94 Jones H O and Brewer J I 1939 Arterial phenomena associated with uterine bleeding in tubal pregnancy Am J Obst & Gynec 38 839-61
95 Jones H W Wade R and Goldberg B 1952 Phosphate liberation by endometrium in presence of adenosinetriphosphate Am J Obst & Gynec 64 1118 24
96 —— 1952 Biochemical and histochemical alkaline glycerophosphatase in normal endometrium endometrial hyperplasia and adenocarcinoma Am J Obst & Gynec 64 1364 7
97 Kaiser I H 1948 Failure of massive doses of estrogen to promote growth of the endometrial coiled arterioles Endocrinology 43 127 32
98 —— 1948 Failure of prostigmin to affect uterine bleeding in rhesus monkey Am J Obst & Gynec 56 664 72
99 —— 1947 Histological appearance of coiled arterioles in endometrium of rhesus monkey baboon chimpanzee and gibbon Anat Rec 99 199 225
100 —— 1947 Absence of coiled arterioles in the endometrium of menstruating new world monkeys Anat Rec 99 353 67
101 Kalant N Pattee C J Simpson G W and Hendelman M 1956 Timing of ovulation Fertil & Steril 7 57 65
102 Keettel W C and Stein R J 1951 The viability of the castoff menstrual endometrium Am J Obst & Gynec 61 440 42
103 Keiffer H 1933 Le probleme du circuit sanguin et de l'hemostase spontanee de l uterus humain Bull Acad Roy Med Belgique S 5 13 253 70
104 King J L 1926 Menstrual records and vaginal smears in a selected group of normal women Contrib Embryol 18 79 94
105 Klopper A 1956 The excretion of urinary pregnanediol during the normal menstrual cycle J Endocrinol 14 xxxii
106 Krohn P L 1954 Cyclic intermenstrual pain the mittelschmerz J Clin Endocrinol 14 682-4
107 —— 1949 Periodic uterine bleeding in ovariectomized monkeys given constant daily doses of oestrogen Endocrinology 45 537-43
108 Krohn P L 1955 The induction of cyclic uterine bleeding in normal and spayed Rhesus monkeys by progesterone J Endocrinol 12 69 85
109 Macht D I 1943 Further historical and experimental studies on menstrual toxin Am J M Sci 206 281-305
110 Markee J E 1939 Menstruation in intraocular endometrial transplants in rhesus monkeys Contrib Embryol 28 219 308
111 —— 1946 Morphological basis for menstrual bleeding Anat Rec 94 481
112 —— 1948 Morphological basis for menstrual bleeding relation of regression to initiation of bleeding Bull New York Acad Med 24 253-68
113 —— 1950 Relation of Blood Flow to Endometrial Growth and the Inception of Menstruation in Engle E T Menstruation and Its Disorders Springfield Ill Thomas pp 165-85
114 —— 1951 The Physiology of Menstruation Seminar Sharp & Dohme vol. 13 pp 3 27
115 Markee J E and Berg B 1944 Cyclic fluctuations in blood estrogen as possible cause of menstruation Stanford M Bull 2 55-60
116 Markee J E Davis J H and Hinsey J C 1936 Uterine bleeding in spinal monkeys Anat Rec 64 231-45
117 McKay D G 1950 Metachromasia in the endometrium Am J Obst & Gynec 59 875-82
118 McKay D G Hertig A T Bardawil W A and Velardo J T 1956 Histochemical observations on the endometrium I Normal endometrium Obst & Gynec 8 22 39
119 —— 1956 Histochemical observations on the endometrium II Abnormal endometrium Obst & Gynec 8 140 56
120 McLennan C E and Koets P 1953 Chemical study of human endometrium throughout the menstrual cycle West J Surg Obst & Gynec 61 169 75
121 Menkin V 1944 Further studies on leukocytosis-promoting factor and on necrosin in inflammatory exudates Am J M Sci 208 290 97
122 Miller A G 1938 Fertile period in practice five year clinical study Surg Gynec & Obst 66 723-31
123 Nissen H W and Yerkes R M 1943 Reproduction in the chimpanzee Report on forty nine births *Anat Rec 86* 567 78
124 Noyes R W and Haman J O 1953 Accuracy of endometrial dating Correlation of endometrial dating with basal body temperature and menses Fertil & Steril 4 504 17
125 Noyes R W Hertig A T and Rock, J 1950 Dating the endometrial biopsy Fertil & Steril 1 3-25
126 Noyes R W 1956 Uniformity of secretory endometrium Obst & Gynec 7 221 8
127 Numers C von 1942 Über die Zellformen des Stromagewebes der menschlichen Gebarmutterschleimhaut Acta obstet et gynecol Scandinav vol. 22 suppl III pp 1 170
128 Odell L and Fishman W H 1950 Studies on beta glucuronidase I Activities in human endometrium Am J Obst & Gynec 59 200 203
129 Okkels H 1950 Histophysiology of Human Endometrium in Engle E T Menstruation and Its Disorders Springfield Ill Thomas pp 139-63
130 Okkels H and Engle E T 1938 Studies on finer

structure of uterine blood vessels of macacus monkey Acta path et microbiol Scandinav 15 150 68
131 O Leary J L 1929 Form changes in the human uterine gland during the menstrual cycle and in early pregnancy Am J Anat 43 269 346
132 Page E Q Glendening M B and Parkinson D 1951 Cyclic biochemical changes in the human endometrium With special reference to the fibrinolytic enzyme Am J Obst & Gynec 62 1100 1105
133 Papanicolaou G N Traut H F and Marchetti A A 1948 Epithelia of Women s Reproductive Organs Correlative Study of Cyclic Changes New York Commonwealth Fund
134 Payne H W and Latour J P A 1955 Quantitative estimations of endometrial glycogen using the anthrone method J Clin Endocrinol & Metab 15 1106-15
135 Phelps D H 1946 Experimental production of menstrual anomalies Endocrinology 39 105-19
136 —— 1947 The factor of previous treatment in experimental menstruation J Clin Endocrinol 7 611 23
137 —— 1946 Endometrial vascular reactions and the mechanism of nidation Am J Anat 79 167 97
138 Phelps D 1954 Physiology of Menstruation and Ovulation in Glandular Physiology and Therapy ed 5 Chicago A M A pp 162 99
139 Pribor H C 1951 Innervation of the uterus Anat Rec 109 339
140 Ramsey E M 1949 Vascular pattern of endometrium of pregnant rhesus monkey (Macaca mulatta) Contrib Embryol 33 113 47
141 —— 1954 Venous drainage of the placenta of the rhesus monkey (Macaca mulatta) Contrib Embryol 35 151 73
142 —— 1956 Circulation in the maternal placenta of the rhesus monkey and man with observations on the marginal lakes Am J Anat 98 159 76
143 Reynolds S R M 1947 The physiologic basis of menstruation A summary of current concepts J A M A 135 552 7
144 —— 1947 Uterine accommodation of the products of conception—physiologic considerations Am J Obst & Gynec 53 901 13
145 —— 1949 Physiology of the Uterus ed 2 New York Paul B Hoeber
146 Roberts Sidney and Szego C M 1953 Steroid interaction in the metabolism of reproductive target organs Physiol Rev 33 593 629
147 Rock J and Hertig A T 1944 Information regarding time of human ovulation derived from study of 3 unfertilized and 11 fertilized ova Am J Obst & Gynec 47 343 56
148 —— 1948 Human conceptus during first two weeks of gestation Am J Obst & Gynec 55 6 17
149 Rogers J and Sturgis S H 1950 Pregnanediol excretion in normal women J Clin Endocrinol 10 89 100
150 Rossman I 1941 Cyclic changes in the endometrial lipins of the rhesus monkey Am J Anat 69 187 227
151 Rossman I and Bartelmez G W 1946 Delayed ovulation a significant factor in the variability of the menstrual cycle Am J Obst & Gynec 52 28 33
152 Roth O A and Burger H 1951 Uber die Bedeutung der Vaginalsmearmethod nach Papanicolaou zur Bestimmung des Ovulationstermins im Vergleich zur Pregnandiolausscheidung und Morgentemperaturkurve Zentralbl f Gynak 73 931 9
153 Rubenstein B B 1950 Endocrine Factors in Sterility of Women in Soskin S Progress in Clinical Endocrinology New York Grune pp 3ol 7
154 Saito O 1926 Beitrage zum Studium der Uterusgefasse Okayama Igakkai Zasshi vol 1926 p 470
155 Sampson J A 1913 Influence of myomata on blood supply of uterus with special reference to abnormal uterine bleeding based on study of 150 injected uteri containing these tumors Surg Gynec & Obst 16 144 80
156 Schlegel J U 1945-46 Arteriovenous anastomoses in endometrium in man Acta anat 1 284 325
157 Schwarz O H and Sherman A I 1950 Hyperplasia of endometrium its relationship to hypertrophy and hyperplasia of uterine blood vessels Am J Obst & Gynec 59 1330-45
158 Shettles L B 1951 The polysaccharide composition of human cervical mucus Fertil & Steril 2 361 8
159 Siegler S L 1952 Treatment of sterility resulting from anovulatory menstruation Fertil & Steril 3 460-99
160 Siegler S L and Siegler A M 1951 Evaluation of the basal body temperature An analysis of 1017 basal body temperature recordings Fertil & Steril 2 287-301
161 Smith O W 1950 Menstrual Toxin—Experimental Studies in Engle E T Menstruation and Its Disorders Springfield Ill Thomas pp 187 205
162 Smith O W and Smith G V 1945 Evidence that menstrual toxin and canine necrosin are identical Proc Soc Exper Biol & Med 59 116 19
163 Smith P E and Engle E T 1927 Experimental evidence regarding role of anterior pituitary in development and regulation of genital system Am J Anat 40 159 217
164 Stein R J and Stuermer V M 1951 Cytodynamic properties of the human endometrium III Variations in the nucleoprotein content of the endometrium during the menstrual cycle Am J Obst & Gynec 61 414 17
165 Stieve H 1943 Weitere Tatsachen Zur Klarung der Frage Wann wird das Ei aus dem Eierstock ausgestossen? Zentralbl f Gynak 67 58 77
166 Stuermer V M and Stein R J 1951 Cytodynamic properties of human endometrium IV Water content of human endometrium during the various phases of the menstrual cycle Am J Obst & Gynec 61 668 9
167 —— 1952 Cytodynamic properties of the human endometrium V Metabolism and the enzymatic activity of the human endometrium during the menstrual cycle Am J Obst & Gynec 63 359 70
168 Sturgis S H and Pommerenke W T 1950 The clinical signs of ovulation A survey of opinions Fertil & Steril 1 113 22
169 Szego Clara M and Roberts Sidney 1953 Steroid action and interaction in uterine metabolism Recent Progress in Hormone Res 8 419 69
170 Szymanowicz J 1922 Observations sur les conditions de la proliferation des glandes uterines chez la femme Gynec et Obstet 5 129 39
171 Telfer M A 1953 Influence of estradiol on nucleic acids respiratory enzymes and the distribution of nitrogen in the rat uterus Arch Biochem 44 111 19
172 Tompkins P 1945 Symposium on recent advances in gynecology and obstetrics timing of ovulation

by basal temperature graphs M Clin North America 29 14-5-34
173 Tompkins P 1945 Basal body temperature graphs as an index to ovulation J Obst & Gynec Brit Emp 52 241 52
174 Topkins P 1953 Endometrial biopsy determination of incidence of ovulation in 402 regularly menstruating women Fertil & Steril 4 76-9
175 Udesky I C 1950 Ovulation in lactating women. Am J Obst & Gynec 59 843-51
176 van der Horst C. J and Gillman, J 1941 The menstrual cycle in elephantulus S Afr J M Sci 6 27-47
177 Van Dyke H B and Ch en G 1936 Observations on the biochemistry of the genital tract of the female macaque particularly during the menstrual cycle Am J Anat 58 473-99
178 —— 1940 The distribution of lipoids in the genital tract of the monkey at different stages of the menstrual cycle Am J Anat 66 411 27
179 Van Herwerden M 1906 Beitrag zur Kenntnis des menstruellen Cyklus Monatschr f Geburtsh u. Gynak 24 730-48
180 Van Wagenen G and Aberle S B D 1931 Menstruation in Pithecus (Macacus) rhesus following bilateral and unilateral ovariectomy performed early in the cycle Am J Physiol 99 271 8
181 Van Wagenen G and Zuckerman S 1933 Uterine bleeding of monkeys in relation to neural and vascular processes II Spinal cord transection and the oestrin level Am J Physiol 106 416-22
182 Velardo J T 1954 Inhibitory action of progesterone and aminopterin on estradiol increased alkaline phosphatase activity in the reproductive tracts of ovariectomized rats Anat Rec (suppl) 120 770
183 Viergiver E and Pommerenke W T 1946 Cyclic variations in viscosity of cervical mucus and its correlations with amount of secretion and basal temperature Am J Obst & Gynec 51 192 200
184 Vollman U 1940 Untersuchungen uber die Korpertemperatur der Frau in Korrelation zu den Phasen ihres Genitalzyklus Monatschr f Geburtsh u Gynak 111 121 53
185 Wade R and Jones H W Jr 1954 Inhibition of human endometrial adenosine triphosphatase by progesterone Obst & Gynec 3 608-14
186 de Watteville H 1951 Pregnanediol determinations in the clinic and in research J Clin Endocrinol 11 251 66
187 Whitelaw M J 1952 Hormonal control of the basal body temperature pattern Fertil & Steril 3 230-44
188 Williams Maude F 1948 The vascular architecture of the rat uterus as influenced by estrogen and progesterone Am J Anat 83 247-307
189 Wislocki G B Bunting H and Dempsey E W 1950 The Chemical Histology of the Human Uterine Cervix with Supplementary Notes on the Endometrium in Engle E T Menstruation and Its Disorders Springfield Ill Thomas pp 23-51
190 Wislocki G B and Dempsey E W 1945 Histochemical reactions of endometrium in pregnancy Am J Anat 77 365-403
191 —— 1948 The chemical histology of the human placenta and decidua with reference to mucopolysaccharides glycogen lipid and acid phosphatase Am J Anat 83 1-41
192 —— 1939 Remarks on lymphatics of reproductive tract of female rhesus monkey (Macaca mulatta) Anat Rec 75 341-63
193 Wislocki G B and Streeter G L 1938 On the placentation of the macaque (Macaca mulatta) from the time of implantation until the formation of the definitive placenta Contrib Embryol 27 1 66
194 Wong A S H Engle E T and Buxton C L 1950 Anovulatory menstruation in women Am J Obst & Gynec 60 790-97
195 Young W C and Yerkes R M 1943 Factors influencing the reproductive cycle in the chimpanzee the period of adolescent sterility and related problems Endocrinology 33 121-54
196 Zuckerman S 1937 Menstrual cycle of primates X Oestrone threshold of uterus of rhesus monkey Proc Roy Soc London s B 123 441 56
197 —— 1941 Effect of previous on subsequent responses of rhesus monkeys to oestrogens J Endocrinol 2 438-43
198 —— 1941 Periodic uterine bleeding in spayed rhesus monkeys injected daily with constant threshold dose of oestrone J Endocrinol 2 263-7
199 —— 1949 Neural factors in the menstrual cycle J Endocrinol 6 xx xxii (Abst)
200 Zuckerman S and Parkes A S 1932 The menstrual cycle of the primates Part V The cycle of the baboon. Proc Zool. Soc London pt 1 139 91
201 Zondek B 1953 Does menstrual blood contain a specific toxin? Am J Obst & Gynec 65 1065-8
202 Zondek, B and Hestrin S 1947 Phosphorylase activity in human endometrium Am J Obst & Gynec 54 173-5
203 Zondek B and Rozin, S 1954 Cervical mucus arborization its use in the determination of corpus luteum function Obst & Gynec 3 463-70

IV

Hormone Changes in Pregnancy

HERBERT S. KUPPERMAN, M.D., PH.D., AND JEANNE A. EPSTEIN, M.D.

THE CHANGES *induced in the maternal organism by* the products of conception are multiple and varied and even today are not yet fully characterized. The *endocrine alterations first observed can be attributed* to the increase in hormonal function of the maternal glandular system during the early part of pregnancy. *Later this activity is transferred to the trophoblast.* Hormone production of the latter organ soon supplements and in some instances will eventually displace or supersede that produced by the maternal organism. Physiologically, the function of the ovaries during the early part of pregnancy is to produce the supply of steroidal substances necessary to induce the decidual changes so important for nidation of the ovum. With the development of the trophoblast as gestation progresses, these same steroids, together with many different tropic hormones, are produced in increasing amounts by the placenta. The productive capacity of the trophoblast throughout various stages of pregnancy is truly amazing. Some of the hormone products secreted, when measured throughout the period of gestation, have been shown to increase at a linear rate until they reach a maximum level just before parturition. Others, particularly the gonadotropic hormone, reach their peak in the midportion of the first trimester. The hormonal alterations of pregnancy are further complicated by the presence of the fetus which eventually, in its own right, has the potentiality of producing both steroidal and tropic hormones that may influence the level of secretion and excretion of these substances by both the maternal organism and placenta, either through stimulatory or inhibitory mechanisms.

Although we now appreciate the potential of the placenta as a production factory of hormonal substances, its endocrine nature was only initially suspected after the classical work of Aschheim and Zondek.[1] The gonadotropic hormone was the first hormone identified as of possible placental origin. It is this substance which provided the physiologic basis for the first biologic test for pregnancy originally described by Aschheim and Zondek. Their monumental discovery gave *endocrinology a much* needed *lift* and established the placenta as an endocrine organ. In subsequent years other hormones have been identified or isolated from the placenta. These have included the estrogens—primarily estradiol, estrone and estriol. Later, progesterone was isolated, and its excretory product, pregnanediol, identified in the urine. Other metabolites of progesterone, such as allopregnanediol and allopregnanolone, have also been identified in the urine of the pregnant female. In addition to the production of the sex steroidal hormones by the placenta, evidence has been presented to indicate that this organ is a rich source of the adrenocortical hormones.[8, 15] These have included the 11-hydroxycorticoids primarily affecting carbohydrate metabolism. More recent data indicate that the salt metabolizing hormone, aldosterone, may also be secreted in significantly increased amounts as pregnancy progresses.[30] Measurable amounts of adrenocorticotropic hormone have been isolated from the placenta in more recent years.[30, 4] The placental production of ACTH was noted to be so excessive that at one time it was felt by commercial manufacturers that the supply of ACTH extracted from the adenohypophyses of animal origin might be adequately augmented by the ACTH extracted from human placental tissue.

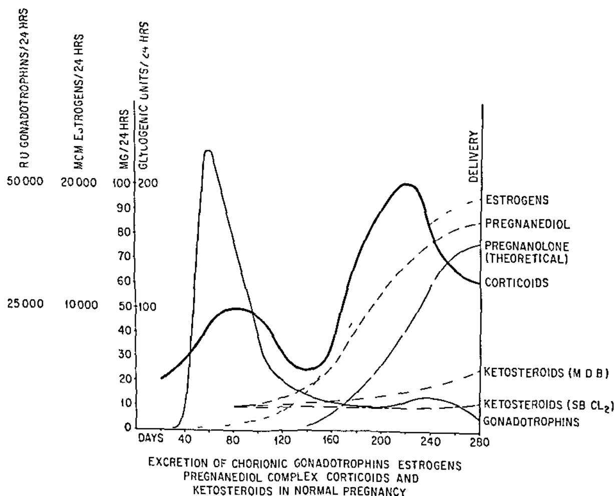
Indirect evidence for the secretion of thyrotropic hormone by the placenta has been made available.[3, 16] This tropic hormone may be a factor responsible in part for the hyperplasia and hypertrophy of the thyroid gland frequently noted in the pregnant female.

CHORIONIC GONADOTROPIN

The most extensively studied hormone produced by the placenta is *the chorionic gonadotropic hormone.* This hormone was the initial substance of an endocrine nature that was isolated from placental tissue. *Its presence in the urine* has served as the basis for the various pregnancy tests that have been devised since the work of Aschheim and Zondek.[1] *The gonadotropic* hormone increases rapidly in titer

shortly after implantation of the ovum and may be detected by one of the many different biological tests[24] as early as the 24th day of pregnancy or approximately 7 10 days after the anticipated day of the missed menstrual period The urinary gonado tropic hormone of pregnancy is characterized pri marily by luteinizing and luteotropic activity This hormone has been incorrectly referred to as the anterior pituitary like hormone of pregnancy It dif fers from the anterior pituitary gonadotropins very markedly in that it possesses relatively little fol licle stimulating hormonal activity When the cho rionic gonadotropic hormone is administered in ade quate amounts to hypophysectomized rats only slight ovarian stimulation is achieved unless very high doses are administered On the other hand the chorionic gonadotropic hormone will synergize with follcile stimulating hormone preparations of pitui tary origin to induce a more rapid and larger in crease in ovarian size associated with luteinization than that noted with FSH alone The luteotropic qualities of the chorionic gonadotropic hormone have been demonstrated by many and its ability to maintain the function of the corpora lutea has been shown both clinically and in experimental studies[2] The luteinizing and luteotropic properties of the chorionic gonadotropic hormone are the ones respon sible for inducing the various end points used in the different tests for pregnancy[26]

The classical Aschheim Zondek procedure is de pendent upon the ability of the chorionic gonado tropic hormone to produce *blut punkten* or *corpora hemorrhagica* in the ovaries of the immature female mouse after the urine or extract has been injected for a period of four days More rapid modifications of this test have been devised whereby instead of *blut punkten* in the mouse the same response in the rabbit was employed as an end point for a posi tive test[13] The rabbit test required only 24 48 hours for completion The procedure was shortened still more when it was noted that ovarian hyperemia could be induced in the rat as a result of chorionic gonadotropic administration[24] This end point has been shown to be a specific one for the luteinizing



EXCRETION OF CHORIONIC GONADOTROPHINS ESTROGENS PREGNANEDIOL COMPLEX CORTICOIDS AND KETOSTEROIDS IN NORMAL PREGNANCY

FIG 1 HORMONE LEVELS DURING PREGNANCY
(*After Venning*)

and luteotropic hormones[26] Follicle stimulating hormone alone does not induce a positive ovarian hyperemic response However FSH may enhance the ovarian hyperemic response to LH or luteotropic hormone Ovarian hyperemia has been used as the end point for the more rapid biological tests for pregnancy The degree of accuracy of this test at times has been reported to exceed or at other times to be considerably below the accuracy reported for the Aschheim Zondek procedure More recently the luteinizing properties of chorionic gonadotropin have been used to induce ovulatory changes in different species of frogs[37] Evidence of a positive test would be manifested by the ability of the preparation to induce an excessive extrusion of eggs in the aqueous substrate in which the animals live The chorionic gonadotropin also has the property of inducing expulsion of sperm from the amphibian testes The latter phenomenon has now become the most popular laboratory end point employed for the detection of pregnancy by biological tests[34]

By using any one of the above different animals and techniques it has been shown that the chorionic gonadotropic hormone reaches detectable levels in the unconcentrated urine specimen at about the 21st 25th day of pregnancy The peak in gonadotropin titer is attained rapidly by approximately the 50th 60th day of pregnancy and then rapidly subsides to a low level maintained from the beginning of the second trimester to term (Fig 1) Preceding parturition a further drop in the hormonal level occurs so that at the time of parturition and up to one week later very minimal levels of chorionic gonadotropin are present After seven days post partum usually no chorionic gonadotropin can be detected in the urine of the postparturient female providing there is no evidence of retained placenta

It is conceivable that the need and function of the chorionic gonadotropin early in pregnancy are primarily for adequate ovarian stimulation to maintain the function of corpus luteum of pregnancy When the function of the corpus luteum is taken over by the placenta there appears to be little need for further chorionic gonadotropic activity Perhaps this then explains the rapid decrease that is noted in chorionic gonadotropin levels at the time of the latter portion of the first trimester of pregnancy

While there is no definitive proof as to the pla



Fig 2a Villi from placenta of first trimester Note well defined cytotrophoblast layer



FIG 2b VILLI FROM PLACENTA IN THIRD TRIMESTER Only syncytial trophoblast is observed

cental cellular source of chorionic gonadotropin that is whether it is produced by the cytotrophoblast or the syncytial trophoblast or both it appears that the cytotrophoblast is the probable source of production of the chorionic gonadotropic hormone This is based in part upon the fact that the fluctuations in secretion and excretion of the chorionic gonadotropin coincides with the increase and decrease in cytotrophoblastic activity The cells of the cytotrophoblast gradually decrease after the first trimester of pregnancy coinciding with the decreasing titer of gonadotropic hormone The histologic picture of placental villi in the first and third trimester are depicted in Figure 2 a and b respectively Note the increased number of cytotrophoblasts in the former compared to those present in the placenta of the third trimester In addition work done by Gey *et al* and Stewart *et al* in tissue culture studies [13 23 44] has shown that it is the cytotrophoblast rather than the syncytium which survives in this artificial environment They have been able to show chorionic gonadotropic hormone activity *in vitro* in tissue cultures of such material consisting primarily of Langhans cells Further data that the cytotrophoblast is the probable source of chorionic gonadotropic hormone are based upon negative evidence using cytochemical techniques which have failed to show fat or steroidal substance present in the Langhans cells [9 5] In addition these cells show evidence of little if any glycogen deposition The presence of these chemical substances would implicate this cellular layer as the source of hormones other than those of protein nature as are the chorionic gonadotropic substances While protein synthesis apparently takes place in both the cytotrophoblast and syncytial trophoblast synthesis in the syncytium is concerned primarily with the production of fetal blood plasma and the cytotrophoblast presumably is concerned with the production of tropic hormones such as chorionic gonadotropin [32]

Evidence has been presented to indicate that the syncytium is stimulated by the hormone produced by the cytotrophoblast to produce increasing amounts of steroid hormone Although there is no specific data to indicate the factors or agents responsible for controlling the level of endogenous gonadotropic hormone secretion from the placenta the role of steroidal hormones in controlling gonadotropic hor



FIG 3 HYDATIDIFORM MOLE (GRADE 3) Cytotrophoblast is primarily noted

mone secretion has been garnered from data obtained in patients with toxemia of pregnancy in whom enhanced or increased levels of chorionic gonadotropin have been demonstrated [39, 40] In these patients the elevated levels of the tropic hormone have been suppressed by the administration of high doses of estrogenic substances [40, 43] Whether this is indicative of interdependence between the gonadotropic hormone from the cytotrophoblast and estrogens is indeterminate at the present time This interrelationship between the steroids and chorionic gonadotropin may be comparable to that noted for the pituitary tropic hormones and their dependent steroidal substances

Further evidence implicating the cytotrophoblastic tissue as the source of chorionic gonadotropic hormone may be noted in the hydatidiform mole and chorioepithelioma Occasional increased levels of progesterone metabolites may be noted in patients with hydatidiform moles This may be ascribed to the ability of the chorionic gonadotropic hormone titer to stimulate ovarian function and maintain corpora luteal activity in these patients In the patient with a chorioepithelioma although very small amounts of estrogen and progesterone metabolites are excreted in their urine enormous amounts of urinary chorionic gonadotropin are usually found As a matter of fact the unusually high titers of gonadotropic hormone obtained are usually pathognomonic of either type of neoplastic process Inasmuch as these neoplasms consist primarily of cytotrophoblast (Fig 3) it may be assumed then that the cytotrophoblast secretes little if any of the steroid hormones and is the principal source of the markedly increased level of chorionic gonadotropic hormone

Quantitative determinations of the actual level of chorionic gonadotropic hormone may be of prime importance in detecting the presence or absence of abnormally elevated titers The interpretation of these titers with respect to international units at times may be difficult because of the lack of a uniform reference standard in the various laboratory techniques Extensive use of the ovarian hyperemia rat pregnancy test in this laboratory has provided a relatively simple method for determining the quantitative hormone level in the serum or urine of the pregnant patient After the establishment of the fact

that an increased gonadotropic titer is present the following procedure is employed. The patient's serum (preferred) or first morning urine sample is diluted by the addition of physiologic saline solution and the highest dilution able to induce ovarian hyperemia is ascertained. All solutions are administered subcutaneously in 2 ml volume and the animals are sacrificed by ether asphyxiation 6-18 hours after the initial injection. Prolonging the time between injections and autopsy from 6 to 18 hours does not appreciably alter the results. A positive test for normal pregnancies will range from a 1:2 to 1:80 titer with normal pregnancies rarely exceeding a positive response in a 1:40 dilution. This is usually obtained at about the 6th-7th week of pregnancy. Since the gonadotropin concentration rapidly falls after the 50th-60th day of pregnancy, undiluted serum or urine is usually required to achieve a positive effect at this time. Hydatidiform moles will give a positive response in dilution ranges from 1:80 to 1:200. A positive response in dilution exceeding 1:250 or 1:300 is considered as diagnostic of the presence of a chorioepithelioma.

In order to provide a comparison between the findings above and international units, the ovarian hyperemia unit was determined for purified chorionic gonadotropin. This value was established by the data obtained from Table 1. It may be noted from these data that the ovarian hyperemia unit is approximately equal to 7.5 international units of chorionic gonadotropin (A.P.L. Ayerst). Hyperemia was observed in all of the animals at the 12.5 international unit level and in none at the 5 international unit dose. Most of the animals showed ovarian hyperemia at the 10 international unit dose. All dilution studies were obtained by the administration of the gonadotropin in 2 cc. of diluent.

At one time it was suggested that the concentration or level of luteinizing hormone could be correlated with the sex of the fetus.[9] Study of the concentration of chorionic gonadotropic hormone, using the dilution technique described above, did not result in a significant correlation between the sex of the fetus and the gonadotropic hormone concentration during the various stages of pregnancy.

Chorionic gonadotropin has also been demonstrated in increased levels in the pregnant mare (PMS). This, however, differs from the gonadotropin of the human in that it is primarily follicle stimulating in activity with only small amounts of LH present. The prime property of extracts prepared from the serum of pregnant mares is that of intense follicular stimulation associated with minimal luteinizing changes. Another property of PMS differing from that of human chorionic gonadotropin is the relatively large molecular size of the former. This is responsible for the failure of the hormone to be

Table 1. RELATIONSHIPS BETWEEN CHORIONIC GONADOTROPIN INTERNATIONAL UNITS AND OVARIAN HYPEREMIA RESPONSE

Dose in international units	Number of animals	Animals with hyperemia	
		Number	Per cent
20	6	6	100
12.5	10	10	100
10.0	10	7 (2)	70 (20)
7.5	10	2 (2)	20 (20)
5	10	0	0

Note: Figures in parentheses = additional number of animals showing submaximal hyperemia.

excreted in the urine of horses. In addition, therapeutic administration of this gonadotropin in the human results in more prolonged effects than are usually noted after human chorionic gonadotropins and thereby requires less frequent injections.

ESTROGENS

The estrogenic compounds are the second major type of hormonal substance produced by the placenta. In the early stages of pregnancy the ovary must be considered as the source of estrogens excreted in the urine in increasing amounts. However, after the placenta is formed, it rapidly takes over the secretion of estrogenic substances coincidental with subsidence of ovarian function. During this transition period the predominant estrogen in the urine changes from estradiol to estriol.[39, 0, 4] The estrogen produced by the ovary is estradiol and is first excreted in increasing amounts in the urine; later the level of this particular type of estrogen is diminished and finally held in abeyance after placental production of estrogenic substances is established. From this point on in pregnancy, estradiol is present in only small amounts in the urine. When the placenta assumes a more dominant role in the second trimester, a marked increase in excretion of estriol and estrone occurs. The urinary levels of estriol and estrone increase progressively during the succeeding months of pregnancy (Fig. 1). Approximately 90 per cent of the estrogens in the urine of a pregnant woman are conjugated in the form of sodium estriol glucuronide. Some unconjugated or free estrogens have also been found in small amounts throughout pregnancy, but are noticeably increased during the terminal phases of pregnancy and during labor.[5, 39, 4] The presence of the free estrogens has been ascribed to hydrolysis of estriol glucuronide by the enzyme glucuronidase

present in the amniotic fluid and blood clots which may be prevalent at the time labor takes place. Premature rupture of the amniotic sac would be conducive to the elaboration and activation of such enzymes. It is the estriol which increases rapidly as pregnancy progresses and as noted above accounts for somewhat more than 90 per cent of the estrogen excreted during the last half of gestation. Both estrone and estradiol appear to increase until about the 25th week of gestation after which time the estradiol level falls rapidly and estrone values appear to fluctuate without any definite pattern (Fig 1). After delivery of the placenta the estrogen level is rapidly cleared from the urine so that the titers fall to nonpregnant levels in about 4 7 days. The site of production of this steroid appears to be predominantly in the syncytial trophoblast rather than in the cytotrophoblast. The evidence for this has been based upon the demonstration of fatty substances and steroidal material by histochemical procedures.[9,5]

The role of estrogen in pregnancy has been a debatable one and one in which there is no unanimity of opinion. There are those who claim that estrogens are important in the metabolism of progesterone. Some believe that estriol may actually be the parent estrogenic substance rather than 17 beta estradiol. If estriol were the primary placental estrogen this would be contrary to what is known about estrogen metabolism. There is no definite experimental evidence to indicate that estriol can be converted to estrone or estradiol. On the other hand the reverse is readily demonstrable. Inasmuch as both estriol and estrone have been identified in the urine and placentae of pregnant women it is possible that the high level of estriol represents a rapid utilization and breakdown of estradiol to estrone and to estriol. This may be an important factor responsible for the efficient utilization of progesterone. This interrelation between estradiol and estriol may be more complex than indicated above. For example estriol will diminish the uterine growth response to estradiol which may represent a competitive inhibition between the two compounds.[0,51] Thus the level of estriol during the later stages of pregnancy may be a measure of the ability of the organism to alter its response to estradiol.

PROGESTERONE

The second of the steroid triumvirate of estrogen progestins and corticoids—the progestins—has been indicted as playing an ever increasingly important role in the homeostatic mechanisms of pregnancy. The secretion of progesterone by the placenta has been established indirectly through the increasing levels of urinary pregnanediol excretion noted from the early stages of pregnancy up to parturition. Whether the earlier levels of pregnanediol excretion the metabolic breakdown product of progesterone may be ascribed to progesterone products by the corpus luteum of pregnancy itself or due in part to progesterone elaboration by the placenta is immaterial. However after the first trimester the placenta is no doubt the principal source of progestational steroids. The progesterone metabolites which have been mentioned above including pregnanediol allopregnanolone and allopregnanediol have all been isolated and identified in adequate amounts from the placenta. On the basis of histochemical techniques it appears that this steroid as is true for the estrogens is produced by the syncytial trophoblast rather than the cytotrophoblast. When pregnanediol excretion levels are used as the index of progesterone synthesis by the placenta the rate of production of progesterone very closely parallels that of estrogen.[4,8] When actual progesterone levels in the blood stream are ascertained utilizing the method of Hooker *et al* the concentrations of conjugated progesterone are usually less than 0 6 μg/cc.[11,21] Free progesterone is relatively low and does not exceed 2 μg/cc.[11] There is no evidence of a progressive increase in blood levels of progesterone as measured by the Hooker technique during the later phases of pregnancy as might be anticipated from a study of pregnanediol levels alone. The observation that the progesterone level in the blood stream does not increase progressively and that the elevated body temperature noted during the luteal phase of the cycle is not maintained after the first trimester of pregnancy has led to speculation concerning the site of progesterone production and its fate during pregnancy. The fall in basal body temperature together with failure to demonstrate a progressive increase in progesterone levels in the blood may be attributed in part to the subsidence of function of the corpus luteum of pregnancy during the first trimester. In addition the failure to demonstrate increasing levels of progesterone in the second and third trimester may be due to the fact that progesterone utilization by the placenta is accomplished *in toto* so that despite an apparent increase in production by the placenta more complete utilization rapidly depletes its level in the blood stream. This rapid utilization may also explain the decrease in basal body temperature noted in the second trimester since sufficient circulating progesterone is not available to achieve an adequate hyperthermic effect.

The statement that the placenta produces progesterone is based upon the observation that the level of pregnanediol continues to increase progressively despite the decline of corpus luteal function. Since pregnanediol is one of the metabolites of progesterone its presence in increasing amounts implies increasing production of progesterone by the placenta. Levels of pregnanediol approximate 10 mgm /

month of pregnancy so that by the ninth month 80 to 100 mgm of pregnanediol per 24 hours may be excreted (Fig 1) The pregnanediol level disappears rapidly from the urine following delivery providing there is no retained placenta The pregnanediol levels will approach zero within 3 to 4 days post partum The high progesterone level which has been noted during pregnancy may also be important in the increased production of corticoids [2, 9] which will be considered below

The metabolic breakdown products of progesterone listed above do not possess the biologic activity of progesterone Secretory changes or glycogen deposition in the endometrium have not been observed following the administration of pregnanediol and pregnanolone These steroids are also ineffective in increasing the basal body temperature—a property invariably evident following administration of the parent compound The metabolic breakdown products of progesterone are usually excreted in conjugated form as glucuronides The increased level of progesterone seen during the early stages of pregnancy and the later metabolic evidence (pregnanediol) of its increased production during the later stages of pregnancy may be important nutrient factors in maintaining the existence of the placenta and the products of conception The effect of progesterone upon the placenta and fetus is augmented or synergized by the presence of estrogens Estrogens have the potential of enhancing certain responses of the organism to progesterone For example estrogens will enhance the progestational response of the endometrium in the nonpregnant female Secretory and decidua like changes in the endometrium will be accentuated by the combined administration of both estrogen and progesterone—more so than with progesterone alone The augmentation of progesterone effect by estrogen has been amply described in the experimental production of deciduomata in the castrate female rat [34] The synergism between estrogen and progesterone is also demonstrated upon breasts vulva vagina and cervix It is also likely that the presence of estrogen in combination with progesterone is responsible for the increased elasticity and vascularity of the vagina and vulva Closely associated with these properties of progesterone and estrogen is the increased elasticity of the pelvic organs and relaxation of the pubic symphysis noted at the time of parturition This response may be attributed in part to the production by the placenta of relaxin—a protein nonsteroidal hormone known to be important for the relaxation of the pubic ligaments of certain rodents during parturition [19] While some manifestation of increased elasticity may be a beneficial component with respect to the pelvic ligaments the relaxation or atonia of certain of the excretory organs may be undesirable This has been noted in the case of the lower ureter and lower bowel with the resultant untoward effect of urinary and fecal stasis However these changes may be due not only to an over all combined physiologic effect of estrogen and progesterone but also to the presence of relaxin produced at this time [33]

CORTICOIDS

The third of the steroid triumvirate produced by the placenta—the corticoids—is a more recently recognized placental hormone The evidence for increased excretion of the corticoids during pregnancy has been based upon direct and indirect evidence The indirect data are those that show the Addisonian patient is usually adequately maintained during her pregnancy with much smaller doses of corticoid than would normally be necessary during the prepregnant phase Jailer and others [8, 22, 3] have shown that the Addisonian patient may be *maintained in perfect* equilibrium during pregnancy without replacement

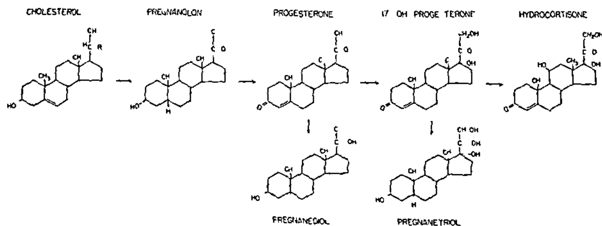


Fig 4 Metabolic pathway from cholesterol to hydrocortisone

therapy as a result of increased endogenous production presumably by the placenta of corticoid substances The corticoid from the placenta may be produced in part from the excessive amount of progesterone substances which are secreted by the syncytial cells of this organ Data by Davis and Plotz and others[8, 8, 17, 45] indicate that progesterone may be an important step in the metabolic pathways involved in the synthesis of corticoids that have been shown to be produced during the various stages of pregnancy Progesterone may be metabolized by the placenta in a similar manner to that which occurs in the adrenal cortex (Fig 4) Thus progesterone is converted by certain metabolic processes to 17 hydroxyprogesterone and then by the process of hydroxylation to 17 hydroxycorticoids This would explain in part the ability of the placenta to produce corticoids in sufficient levels to supplement and maintain adequately the Addisonian or adrenal insufficient individual in the normal homeostatic pattern The drop in urinary corticoids which is noted at the time of parturition may be a reflection of the fact that the progesterone level is also diminished as noted by the decreased pregnanediol excretion Hence the corticoid precursors which had been produced by the active placenta are likewise decreased resulting in a diminished output of corticoid metabolites

In discussing the increase in corticoid excretion during the pregnant state one must also mention the findings in relation to aldosterone excretion throughout pregnancy Aldosterone is the salt retaining steroid which was recently isolated from the adrenal cortex by Simpson and Tait[3] and may be present in one of two isomeric forms (Fig 5) In studies of aldosterone levels during pregnancy it was first noted that there was little if any increase in free aldosterone over levels observed in the non pregnant state[50] Conjugated aldosterone fractions however were increased considerably above the normal level of the nonpregnant female This was noted after the third month when the significant increase was maintained up until term The average output during the last few months of pregnancy was in the range of 25 μg/24 hours Postpartum there is a precipitous drop of the conjugated aldosterone levels to levels noted in the nongravid female (Fig

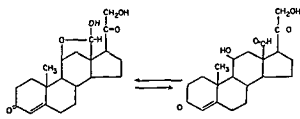
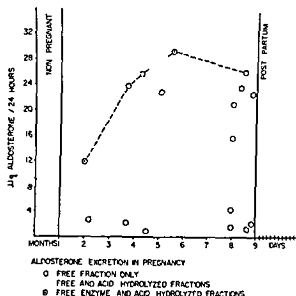


Fig 5



(*After Venning*)
Fig 6

6) Aldosterone levels in pregnancy have shown some interesting results in patients with toxemia of pregnancy Normal patients and those with toxemia of pregnancy showed little difference between their total aldosterone levels However of those subjects studied the free urinary aldosterone level was considerably increased in patients with toxemia of pregnancy[50] The true significance of such findings must be taken in the light of other studies of hormonal patterns during toxemia of pregnancy The total corticoid excretion has been considered to be higher in patients with toxemias compared to the nontoxemic patient Interestingly these same toxemic patients showed a decreased pregnanediol level associated with an increased chorionic gonadotropin In the light of our knowledge of corticoid production from progesterone compounds one can explain the decreased pregnanediol levels and increased corticoid excretion upon the enhanced conversion of the progesterone steroids to corticoids With the decreased level of progestins increased levels of gonadotropin would be anticipated and in fact have been observed[39, 40]

In a discussion of corticoid production and excretion a word about the 17 ketosteroid excretion in the blood and urine of pregnant women is needed The 17 ketosteroids as determined by the Zimmerman reaction are well within the normal limits during pregnancy[4, 8] Data reporting an increase in ketosteroid excretion during the 140th to 160th day of pregnancy may well be attributed to increased amounts of pregnanolone a 20 ketosteroid which may be a contaminant responsible for falsely elevated values when the Zimmermann reaction is used (Fig 1) Use of the antimony trichloride method of Pincus[32] for the color reaction determination of 17

ketosteroids showed no difference in ketosteroid excretion of the pregnant or nonpregnant female regardless of the stage of pregnancy (Fig 1) This latter method accounts for practically all of the normally occurring ketosteroids with the exception of the dehydroisoandrosterone In general the recent experiences in determining keto teroid excretions during pregnancy have indicated that there is no significant increase in ketosteroid excretion in the pregnant female On the other hand a decrease in 17 ketosteroid excretion has been noted in the post partum female This phenomenon may be explained in part by the production of ketosteroids from the fetal adrenal as well as from the placenta With delivery of the fetus one would therefore expect an absolute decrease in 17 ketosteroid excretion levels of the postparturient mother as a result of removal of the auxiliary fetal source of androgens

The innermost zone layer of the adrenal cortex is markedly hypertrophied in the fetus This zone has been referred to as the androgenic zone X zone or provisional zone layer and grows particularly rapidly during the last three months of intra uterine life It degenerates shortly after birth Davis and Plotz [8] showed that the provisional zone is absent or almost completely absent in anencephalic monsters and in infants in whom the hypophyses are unusually small Their experiments with tagged precursors of cholesterol have indicated that a high rate of cholesterol synthesis occurs in the fetal adrenals during the third to fifth month of intra uterine life This synthesis in the adrenal cortex is absent in the anencephalic monsters Relatively large amounts of ketosteroids are found in the urine of the newborn infant during the first two days of life Thereafter this level disappears very rapidly Whether this is due to the temporary carry over of the maternal steroid products or whether it is due to the transient activity of the provisional zone layer of the newborn s adrenal cortex and subsequent atrophy which occurs about this time is difficult to prove These changes in the fetus indicate that the fetal adrenal itself may be a rich source of adrenocortical hormones which may confuse the picture of the steroid excretion pattern in the pregnant female All steroid excretory products in the urine of the pregnant female may not be derived from the placenta alone but may also arise in part from the fetal adrenals

A study of the excretion of a single steroid such as androsterone or etiocholanolone has indicated that androsterone is not isolated from the urine during the last two months of pregnancy Etiocholanolone is reduced to small amounts Davis and Plotz [8] have suggested that the decreased excretion of androsterone and etiocholanolone was due to the fact that production of the adrenocortical precursors was depressed during the latter portion of pregnancy All of the data on excretion of steroid hormones in the urine of the pregnant female of course is complicated by the role of the fetal adrenal cortex as noted previously Davis and his co workers showed that tritium labeled cholesterol which must be considered as the possible precursor of the different steroid hormones passes the human placenta in early and late pregnancy It must also be accepted that the hormones produced by the fetal portion of the placenta can pass into the maternal circulation

ADRENOCORTICOTROPIC HORMONE

In addition to the adrenocortical hormones adrenocorticotropic substances recently have been isolated from human placental tissue [30, 48] Tarantino s studies [48] on bovine placentae show that ACTH activity was present in the chorion but not in the decidua The actual role of this hormone in pregnancy is debatable at the present time Its presence nevertheless has provoked interest as to the role of this particular hormone in the homeostasis of the pregnant female The ACTH noted in the placenta may not actually be due to its production or secretion by this organ but may merely represent storage of ACTH by the placenta Extraction of the adrenocorticotropic hormone from the placenta while yielding substantial levels has been fraught with difficulty and has made this source impractical commercially at the present time The cellular origin of ACTH produced by the placenta is unknown but based upon the protein nature of the hormone in all probability it would be derived from the cytotrophoblast The placental ACTH does not appear to be a prime stimulating factor in the physiology of the fetal adrenal Davis and Plotz [8] have reported a failure of the anencephalic monster to show proper steroid synthesis in its adrenal cortex Apparently lack of fetal hypophyseal ACTH despite adequate levels of placental ACTH is sufficient to prevent adequate fetal adrenal response

THYROTROPIC HORMONE

The production of thyrotropic hormone and/or thyroid hormone by the placenta has been a problem of important clinical context Evidence has been accumulated to indicate that the chorioepithelioma or hydatidiform mole may have the potentiality of producing thyrotropic hormone One such case has been seen in this institution recently where an increase in thyroid function was noted in a patient with a hydatidiform mole Following delivery of the mole increased thyroid function diminished very promptly Assay of the patient s serum according to the method of D Angelo and Gordon [6] revealed an

increased amount of thyrotropic stimulating activity prior to passage of the mole.[31] Other case reports have indicated a similar relationship existing between thyrotropic secretion and placental tissue. Another patient with a hydatidiform mole showed clinical evidence of hyperthyroidism and with removal or passage of the mole the signs and symptoms of hyperthyroidism subsided.[46] Associated with this experimental evidence one should note the well recognized clinical fact that an appreciable increase in the size of the thyroid gland may occur in patients during pregnancy. Whether this is a measure of increased thyroid stimulating hormone activity based upon the placental activity or whether this is a compensatory mechanism due to the increased need for iodine by the developing fetus is difficult to determine at the present time. Evidence of hyperthyroidism has been noted in the patients with neoplastic changes in the placenta which while producing excessive amounts of chorionic gonadotropin may also be producing increased amounts of thyrotropic hormone. An increase in thyroid hormone levels during pregnancy has been suggested by increased amounts of serum precipitable iodine present in pregnancy. The increase is maintained until delivery and appears to be thyroid hormone. In addition to the laboratory findings noted clinical manifestations of hyperthyroidism may also be prevalent during pregnancy. The clinical picture may be confused further by the presence of thyroid nodules. Are these true neoplasms or just an expression of an increased responsiveness of the patient's thyroid to the placental thyrotropic hormone? The therapeutic management of such nodules must be tempered by the fact that they may be temporary in nature and are usually induced as a result of increased endogenous production of TSH not by the patient's pituitary gland but by the placenta. Thyroid enlargement whether smooth or nodular must be treated conservatively unless profound signs of clinical hyperthyroidism are present.

ABNORMAL CONDITIONS OF PREGNANCY

A discussion of hormonal alterations during pregnancy would not be complete without including observations on hormone excretion patterns during abnormal pregnancies. Various studies on toxemia of pregnancy have been published to indicate that an alteration in the normal hormonal excretion pattern during pregnancy may occur.[27, 39, 40, 43, 50] An increase in chorionic gonadotropic hormone has been noted during toxemia of pregnancy.[40, 43] This has also been associated at times with a low pregnanediol excretion. Other data have been reported indicating that an increased amount of salt retaining substance has also been observed during toxemia. This has been particularly true of the urinary free aldosterone assay levels. There has been no evidence of any significant increase of the conjugated aldosterone steroid in toxemia of pregnancy.[50] The role played by the chorionic gonadotropin and/or the salt retaining hormone cannot be properly identified at the present time. However based upon the observation of the increased gonadotropic hormone being a factor in toxemia and the reported finding that estrogen has the potentiality of decreasing the chorionic gonadotropin level[8, 40, 43] treatment with estrogen may diminish the toxic manifestations noted in some patients with pre-eclampsia or toxemia of pregnancy. Estrogen and progesterone may be synergistic in their effect upon diminishing gonadotropin excretion.

The problem of habitual abortion also must be discussed at this time. A multitude of entities or factors has been suggested as being important in the etiology of repeated or habitual abortion. Inasmuch as there is no common etiology for all patients with habitual abortions one must acknowledge that there can be no common therapy for all such patients. Studies professing therapeutic success in this field have been conflicting. Some investigators have shown that administration of estrogens to patients with repeated and habitual abortions may result in an adequate therapeutic salvage. The question of the efficacy of estrogen has been raised in this institution by Greene[16] and by Ferguson.[1] The latter worker in adequately controlled studies has indicated that there is no clear cut difference between the therapeutic efficacy of estrogens or placebos upon fetal salvage in patients with a history of repeated or pending abortions and in those with eclampsia or pre-eclampsia.[10] The premise for the use of estrogen in patients with repeated abortions was based upon the observation that administration of estrogen was associated with an increase in pregnanediol glucuronide excretion.[41, 4] Standardization of techniques revealed that the glucuronide excreted in increased amounts after estrogen therapy was actually the glucuronide of the estrogen administered and not of pregnanediol.[7, 43] Other data has indicated that perhaps the low pregnanediol level may offer a clue to therapy in patients with a history of repeated abortion. Studies done in this laboratory have indicated that in those individuals with a history of repeated abortions in whom a lower than anticipated pregnanediol level is noted for their particular stage of pregnancy oral therapy with high doses of progesterone in the range of 1000 mgm/day will be helpful in promoting pregnancy salvage in many cases.[5] The therapeutic response in these patients has been adequate and promising although 100 per cent salvage is not achieved and only about 25 per cent of all patients with a history of repeated

abortions fit into the category of having a low pregnanediol excretion for their particular stage of pregnancy [5]

In the discussion of abnormal conditions of pregnancy one must also consider the role of the placenta in the release or secretion of pressor amines Increased elaboration of sympathomimetic amines from the placenta has not been noted in normal pregnancies and there is no evidence to show that medullary function of the adrenal can be assumed by the placenta Adrenalin or norepinephrine like substances have not been extracted in sufficient amounts from placental tissue At times nevertheless it does appear as if the symptomatology of toxemia of pregnancy may be akin to other states associated with overproduction of sympathomimetic amines The only clinical material to substantiate increased production of sympathomimetic amines during pregnancy has been the frequent association of pheochromocytoma and pregnancies [14, 8] This does not mean that there is a direct correlation between pregnancy and pheochromocytoma but indicates that the presence of a tumor of the adrenal medulla may be detected or its secretion accentuated during pregnancy In a summary of the world literature in 1955 [14] of 115 female patients with pheochromocytoma between the ages of 20 to 50 years 30 per cent were pregnant

The symptomatology of a patient with a pheochromocytoma may be analogous to that of one with toxemia of pregnancy The diagnosis should be suspected when such symptoms are paroxysmal in nature Their rapid dissolution after administration of 5 mgm phentolamine should be confirmatory proof of the diagnosis of pheochromocytoma Provocative stimulation of a hypertensive attack with histamine or mecholyl while of no great danger in the non pregnant female should not be employed in the pregnant female because of the untoward effect that these agents or the provoked responses might have on the products of conception or uterine motility The diagnosis of the presence of a pheochromocytoma may be established in more definitive terms if a positive test for urinary catecholamines is obtained The presence of these pressor amines in the urine can be considered as being one of the most important diagnostic criteria for the presence of a pheochromocytoma More recently there has been described a biologic test for the presence of the pressor amines utilizing the effect of urine from patients suspected of having the condition upon induced contractions of isolated spiral segments taken from the dorsal aorta of the rabbit [18] Violent contractions have been reported with urine of patients with pheochromocytoma as compared to little or no effect with normal urine when added to the *in vitro* specimen Cardiovascular characteristics noted during the active secretion of the pressor amines may include arrhythmia diffuse changes in the ST segment and T waves and multifocal ventricular extra systoles Runs of nodal tachycardia with a wandering pacemaker have been reported together with occasional sinoatrial block and peaked P wave changes The patient may show the classical signs of vascular collapse which may lead the obstetrician to suspect uterine rupture or a profound intra uterine hemorrhage Vascular collapse may occur following minimal trauma induction of anesthesia or surgery

In addition to the above the patient may also show clinical evidence of hypermetabolism and decreased glucose tolerance both being attributed to overproduction or predominance of epinephrine over that of norepinephrine by the tumor

The apparent increased tendency for these tumors to become manifest during pregnancy may be based upon the inadvertent massage of the tumor by the activity of the fetus during the third trimester of pregnancy While intentional massage of the flank area in patients suspected of having pheochromocytoma has been suggested as a diagnostic test to provoke a hypertensive episode [14] we feel such a technique is contraindicated due to the adverse effect a positive response may have upon the mother and fetus The relatively high incidence of pheochromocytomas in pregnancy demands a high index of suspicion on the part of the attending obstetrician in order to avoid the oft times fatal complications to either mother or child that may result in these cases

BIBLIOGRAPHY

1 Aschheim S and Zondek B 1928 Schwangerschaft adiagnose aus dem Harn (durch Hormonnachweis) Klin Wchnschr 7 8 9

2 Astwood E B 1941 The regulation of corpus luteum function by hypophysial luteotrophin Endocrinol 28 309 20

3 Bongiovanni A M 1953 The detection of pregnanediol and pregnanetriol in the urine of patient with adrenal hyperplasia suppression with cortisone Bull Johns Hopkins Hosp 92 244 51

4 Borsh R and de Watteville H 1952 Hormone assays in obstetrics and gynecology Vitamins & Hormones 10 141 81

5 Cohen S L Marrian G F and Watson M 1935 Excretion of oestrin during pregnancy Lancet 1 674 6

6 D Angelo S A and Gordon A S 1950 Simultaneous detection of thyroid and thyrotropic hormones in vertebrate serum Endocrinol 46 39 54

7 Davis M E and Fugo N W 1950 Steroids in the treatment of early pregnancy complication J A M A 142 778 85

8 Davis M E and Plotz E J 1956 Hormonal interrelationships between maternal adrenal placental and fetal adrenal functions Obs & Gyn Survey 11 1-43

9 Dempsey E W and Wislocki G B 1944 Obser

vations on some histochemical reactions in the human placenta with special reference to the significance of the lipoids glycogen and iron Endocrinol 35 409 29
10 Ferguson J H 1953 Effect of stilbestrol on pregnancy compared to the effect of a placebo Am J Obs & Gyn 65 592 601
11 Forbes T R 1951 Systemic levels of plasma progesterone during pregnancy in women and monkeys Endocrinol 49 218 24
12 Fried P H and Rakoff A E 1952 The effects of chorionic gonadotropin and prolactin on the maintenance of corpus luteum function J Clin Endocrinol & Metab 12 321 37
13 Friedman M H and Lapham M E 1939 A simple rapid procedure for the laboratory diagnosis of early pregnancy Am J Obs & Gyn 38 717 18
14 Gemmell A A 1955 Phaeochromocytoma and the obstetrician J Obs & Gyn Brit Emp 62 195 202
15 Gey G O Seegar G E and Hellman L M 1938 Production of gonadotropic substance (Prolan) by placental cells in tissue culture Science 88 306-7
16 Greene R R Personal communication
17 Hechter O Zaffaroni A Jacobsen R P Levy H Jeanloz R W Schenker V and Pincus G 1951 The nature and the biogenesis of the adrenal secretory product Recent Prog Hormone Res 6 215-46
18 Helmer O and Martz B L 1956 Use of rabbit aortic strip in diagnosis of pheochromocytoma Exhibit A M A June 1956
19 Hisaw F L 1926 Experimental relaxation of the pubic ligament of the guinea pig Proc Soc Exper Biol & Med 23 661 3
20 Hisaw F L Velardo J T and Goolsby C M 1954 Interactions of estrogens on uterine growth J Clin End & Metab 14 1134 43
21 Hooker C W and Forbes T R 1947 Bio assay for minute amounts of progesterone Endocrinol 41 158 69
22 Jailer J W and Knowlton A I 1950 Simulated adreno cortical activity during pregnancy in an Addisonian patient J Clin Invest 29 1430 36
23 Jones G E S Gey G O and Gey M R 1943 Culture of placental tissue—chorionic gonadotropin Bull Johns Hopkins Hosp 72 26 38
24 Kupperman H S and Greenblatt R B 1948 The two hour test for the early diagnosis of pregnancy Postgrad Med 4 519 27
25 Kupperman H S and Lefcovics S 1957 The diagnostic and therapeutic use of progesterone in problems of sterility Fertility & Sterility 8 131 46
26 Kupperman H S McShan W H and Meyer R K 1948 Gonadotrophic hormones and ovarian hyperemia in the rat Endocrinol 43 275 82
27 Lloyd C W Hughes E C Lobotsky J Rienzo J and Avery G M 1952 Some hormone studies in normal and toxemic pregnancy J Clin Invest 31 1056-63
28 Maloney J M 1955 Pheochromocytoma in pregnancy Cesarean section and adrenalectomy N Eng J Med 253 242 3
29 Nieburgs H E and Greenblatt R B 1948 Blood gonadotropin studies during pregnancy in relation to the fetal sex J Clin Endocrinol 8 600
30 Opsahl J C and Long C N H 1951 Identification of ACTH in human placental tissue Yale J Biol Med 24 199 209
31 Phillips J Personal communication
32 Pincus G 1943 New color reaction for certain urinary 17 ketosteroids Endocrinol 32 176 81
33 Pommerenke W T 1934 Experimental ligamentous relaxation in guinea pig pelvis Am J Obs & Gyn 27 708 13
34 Robbins S L and Parker F 1948 The use of the male North American frog (Rana pipiens) in the diagnosis of pregnancy Endocrinol 42 237-43
35 Rolland C F Matthews J D and Matthews C D 1953 Pregnancy in Addison's disease J Obs & Gyn Brit Emp 60 57 60
36 Rothchild I Meyer R K and Spielman M A 1940 A quantitative study of estrogen progesterone interaction in the formation of placentomata in the castrate rat Am J Physiol 128 213 24
37 Shapiro H A and Zwarenstein H 1935 Test for early diagnosis of pregnancy in South African clawed toad (Xenopus laevis) So African Med J 9 202 5
38 Simpson S A and Tait J F 1953 Nature of circulating hormones of adrenal cortex in man Arch Middlesex Hosp 3 209 18
39 Smith G V and Smith O W 1941 Estrogen and progestin metabolism in pregnancy II The endocrine imbalance of pre eclampsia and eclampsia J Clin Endocrinol 1 470 76
40 —— 1941 Estrogen and progestin metabolism in pregnancy III The effect of hormone administration in pre eclampsia J Clin Endocrinol 1 477-84
41 —— 1954 Prophylactic hormone therapy Relation to complications of pregnancy Obs & Gyn 4 129 41
42 Smith O W Smith G V and Hurwitz D 1946 Increased excretion of pregnanediol in pregnancy from diethylstilbestrol with special reference to the prevention of late pregnancy accidents Am J Obs & Gyn 51 411 15
43 Sommerville I F 1950 Observations on the placental sex hormones in the toxaemias of pregnancy In Toxaemias of Pregnancy Ciba Fdn Symp Blakiston Co Philadelphia P 216
44 Stewart H C Sano M E and Montgomery T L 1948 Hormone secretion by human placenta grown in tissue culture J Clin Endocrinol 8 175-88
45 Stone D Gobiel E Feinstein M and Hechter O 1953 Influence of ACTH on progesterone transformation to corticosteroid in perfused bovine adrenals Fed Proc 12 139
46 Studdiford W E Personal communication
47 Sulman F G and Bergman F 1953 Adrenocorticotrophic activity of placental extracts J Obs & Gyn Brit Emp 60 123 5
48 Tarrantino C 1951 Sulla presenza di ACTH nella placenta Folia endocrinol 4 197 201
49 Venning E H 1955 Endocrine changes in normal pregnancy Am J Med 19 721 3
50 Venning E H and Dyenfurth I 1956 Aldosterone excretion in pregnancy J Clin Endocrinol & Metab 16 426 8
51 Wicks A E and Segal S J 1956 Time and dose relationships in estriol estradiol interaction Proc Soc Exp Biol & Med 93 270 73
52 Wislocki G B Dempsey E W and Fawcett D W 1948 Some functional activities of the placental trophoblast In Normal and Pathological Physiology of Pregnancy The Williams and Wilkins Co Baltimore

V

The Anatomy and Endocrinology of the Human Placenta

KERMIT E KRANTZ M D

INTRODUCTION

ALTHOUGH the human placenta is one of the most available organs its physiology and anatomy are poorly understood The history of the ideas and concepts concerning the placenta is fraught with philosophic as well as scientific interpretation In the records of the Veddas a primitive people of Ceylon the placenta was described as being covered with mucus and blood and containing the demons of the obstetrical patient Aristotle several centuries before Christ described the human placenta and also the placental relationships in viviparous fishes

Galen in the second century of the Christian era exhibited a deep interest in embryology He described in detail the chorion and its relationship to the uterus He even went so far as to postulate the origin of the chorion from the semen He noted the vessel pattern of the placenta and umbilical cord and described the independent pulsations of the fetal heart He further noted a relationship between the pulsation of the umbilical cord and the placenta

Leonardo da Vinci [93] pictured the gravid uterus in his notebook carrying over into the human conditions existing in the cotyledonary placentae of ruminants

Vesalius [150] was the first fully to describe and illustrate the gross appearance of the chorion and the amnion Yet the frondose regions of the chorion and uterine wall in apposition lacked a name It was Realdo Columbus [26] in 1559 who first gave the name placenta to the afterbirth even though many give Fallopius [48] the credit for naming the organ Because of the multiplicity of monographs concerning placentation and because placentation in the human is unique in its own right this chapter will be limited primarily to the discussion of the human placenta

Placentation is the necessary process of the formation of a relationship between the maternal uterus and the embryo without which death of the embryo would occur and perpetuation of the species per se would cease In the human a more complex and dependent arrangement occurs than in most species The human placenta is considered *hemachorial* because of the intimate relationship of the villi with maternal blood classified *chorio-allantoic* because of its pattern of vascularization *villous* because of its villi *deciduate* because maternal decidua accompanies it in the process of separation and *discoidal* because of its shape

THE FETAL-MATERNAL VASCULAR RELATIONS

Fabricius [46] claimed there was a direct continuity of blood between the uterus and the placenta with the two sets of vessels being contiguous rather than actually uniting with each other Cheselden stated in his textbook of anatomy that a direct communication exists between the mother and the fetus This communication he thought was a result of anastomoses between the arteries and veins of the uterus with the veins and arteries of the placenta On the other hand others argued that the circulations of the mother and fetus were in direct continuity [62, 8, 50] They felt that the arteries and veins of the uterus were connected with the veins and arteries of the fetus so that the blood could become oxygenated in the lungs of the mother and receive the necessary nutrition to enable the child *in utero* to live and grow William Cowper [30] wrote Hence it appears there is a circulation of blood between mother and fetus and it seems as if the blood vessels of both did germinate and inosculate with each other

The three main theories of direct continuity of fetal and maternal vessels in the placenta that have been held are (1) end to end contiguity of maternal and fetal vessels (2) direct continuity of maternal and fetal vessels and (3) the complex scheme of inosculation of maternal and fetal vessels Evidence was adduced by many observers through *injection studies that there is an absence of any direct continuity between the maternal and fetal circulations* [6, 07, 95, 108, 109, 113, 173]

vations on some histochemical reactions in the human placenta with special reference to the significance of the lipoids glycogen and iron Endocrinol 35 409 29

10 Ferguson J H 1953 Effect of stilbestrol on pregnancy compared to the effect of a placebo Am J Obs & Gyn 65 592 601

11 Forbes T R 1951 Systemic levels of plasma progesterone during pregnancy in women and monkeys Endocrinol 49 218 24

12 Fried P H and Rakoff A E 1952 The effects of chorionic gonadotropin and prolactin on the maintenance of corpus luteum function J Clin Endocrinol & Metab 12 321 37

13 Friedman M H and Lapham M F 1939 A simple rapid procedure for the laboratory diagnosis of early pregnancy Am J Obs & Gyn 38 717 18

14 Gemmell A A 1955 Phaeochromocytoma and the obstetrician J Obs & Gyn Brit Emp 62 195-202

15 Gey G O Seegar G E and Hellman L M 1938 Production of gonadotropic substance (Prolan) by placental cells in tissue culture Science 88 306-7

16 Greene R R Personal communication

17 Hechter O Zaffaroni A Jacobsen R P Levy H Jeanloz R W Schenker V and Pincus G 1951 The nature and the biogenesis of the adrenal secretory product Recent Prog Hormone Res 6 215-46

18 Helmer O and Martz B L 1956 Use of rabbit aortic strip in diagnosis of pheochromocytoma Exhibit A M A June 1956

19 Hisaw F L 1926 Experimental relaxation of the pubic ligament of the guinea pig Proc Soc Exper Biol & Med 23 661 3

20 Hisaw F L Velardo J T and Goolsby C M 1954 Interactions of estrogens on uterine growth J Clin End & Metab 14 1134-43

21 Hooker C W and Forbes T R 1947 Bio assay for minute amounts of progesterone Endocrinol 41 158 69

22 Jailer J W and Knowlton A I 1950 Simulated adreno cortical activity during pregnancy in an Addisonian patient J Clin Invest 29 1430 36

23 Jones G E S Gey G O and Gey M R 1943 Culture of placental tissue—chorionic gonadotropin Bull Johns Hopkins Hosp 72 26 38

24 Kupperman H S and Greenblatt R B 1948 The two hour test for the early diagnosis of pregnancy Postgrad Med 4 519 27

25 Kupperman H S and Lefcovics S 1957 The diagnostic and therapeutic use of progesterone in problems of sterility Fertility & Sterility 8 131-46

26 Kupperman H S McShan W H and Meyer R K 1948 Gonadotrophic hormones and ovarian hyperemia in the rat Endocrinol 43 275 82

27 Lloyd C W Hughes E C Lobotsky J Rienzo J and Avery G M 1952 Some hormone studies in normal and toxemic pregnancy J Clin Invest 31 1056-63

28 Maloney J M 1955 Pheochromocytoma in pregnancy Cesarean section and adrenalectomy N Eng J Med 253 242 3

29 Nieburgs H E and Greenblatt R B 1948 Blood gonadotropin studies during pregnancy in relation to the fetal sex J Clin Endocrinol 8 600

30 Opsahl J C and Long C N H 1951 Identification of ACTH in human placental tissue Yale J Biol Med 24 199 209

31 Phillips J Personal communication

32 Pincus G 1943 New color reaction for certain urinary 17 ketosteroids Endocrinol 32 176-81

33 Pommerenke W T 1934 Experimental ligamentous relaxation in guinea pig pelvis Am J Obs & Gyn. 27 708 13

34 Robbins S L and Parker F 1948 The use of the male North American frog (Rana pipiens) in the diagnosis of pregnancy Endocrinol 42 237 43

35 Rolland C F Matthews J D and Matthews C D 1953 Pregnancy in Addison s disease J Obs & Gyn Brit Emp 60 57 60

36 Rothchild I Meyer R K and Spielman M A 1940 A quantitative study of estrogen progesterone interaction in the formation of placentomata in the castrate rat Am J Physiol 128 213 24

37 Shapiro H A and Zwarenstein H 1935 Test for early diagnosis of pregnancy in South African clawed toad (Xenopus laevis) So African Med J 9 202 5

38 Simpson S A and Tait J F 1953 Nature of circulating hormones of adrenal cortex in man Arch Middlesex Hosp 3 209 18

39 Smith G V and Smith O W 1941 Estrogen and progestin metabolism in pregnancy II The endocrine imbalance of pre eclampsia and eclampsia J Clin Endocrinol 1 470 76

40 —— 1941 Estrogen and progestin metabolism in pregnancy III The effect of hormone administration in pre-eclampsia J Clin Endocrinol 1 477-84

41 —— 1954 Prophylactic hormone therapy Relation to complications of pregnancy Obs & Gyn 4 129 41

42 Smith O W Smith G V and Hurwitz D 1946 Increased excretion of pregnanediol in pregnancy from diethylstilbestrol with special reference to the prevention of late pregnancy accidents Am J Obs & Gyn 51 411 15

43 Sommerville I F 1950 Observations on the placental sex hormones in the toxaemias of pregnancy In Toxaemias of Pregnancy Ciba Fdn Symp Blakiston Co Philadelphia P 216

44 Stewart H C Sano M E and Montgomery T L 1948 Hormone secretion by human placenta grown in tissue culture J Clin Endocrinol 8 175 88

45 Stone D Gobiel E Feinstein M and Hechter O 1953 Influence of ACTH on progesterone transformation to corticosteroid in perfused bovine adrenals Fed Proc 12 139

46 Studdiford W E Personal communication

47 Sulman F G and Bergman F 1953 Adrenocorticotrophic activity of placental extracts J Obs & Gyn Brit Emp 60 123 5

48 Tarrantino C 1951 Sulla presenza di ACTH nella placenta Folia endocrinol 4 197 201

49 Venning E H 1955 Endocrine changes in normal pregnancy Am J Med 19 721 3

50 Venning E H and Dryenfurth I 1956 Aldosterone excretion in pregnancy J Clin Endocrinol & Metab 16 426 8

51 Wicks A E and Segal S J 1956 Time and dose relationships in estriol estradiol interaction Proc Soc Exp Biol & Med 93 270 73

52 Wislocki G B Dempsey E W and Fawcett D W 1948 Some functional activities of the placental trophoblast In Normal and Pathological Physiology of Pregnancy The Williams and Wilkins Co Baltimore

villous space with separate maternal and fetal circulations [1 5 3 5 8 15 84 49 94 6 39 129 87 141 15 151 66 90 25 158 23 136 47 1 1 6] In spite of the overwhelming evidence to the contrary some workers held that no blood was found in the intervillous space [68] No explanation for the absence of blood other than the hypothesis of a uterine milk from the endometrial glands was made This concept was modified by some to occur only at one time in pregnancy This became known as the Hoffman uterine milk theory [72] One worker felt the intervillous space was composed of lymph sinuses [4] As late as 1931 there were authorities on the placenta who doubted that blood normally enters the intervillous space [14]

THE LINING OF THE INTERVILLOUS SPACE

The main ideas that have been associated with the lining of the intervillous space are

1 The uterine arteries and veins open into the placental sinus through which the blood is transmitted to bathe the villi directly without the intermediation of any maternal structure [73 115]
2 The intervillous space represents extravascular clefts or spaces in which the fetal villi hang after erosion of the maternal blood vessels has occurred [49 90 86 66]
3 The intervillous space represents dilated blood vessels arteries capillaries and veins The villi totally in later pregnancy break through the vessel walls to become bathed in blood [151 88 89]
4 The intervillous space represents dilated maternal capillaries and veins [94]
5 The intervillous space represents dilated maternal veins (a) through which the villi have not broken but have only partially indented their walls [1 5] and (b) in which the villi may erode and wear through the walls to become directly bathed by blood [8]
6 The intervillous space represents dilated maternal capillaries (a) the villi may totally break through their walls [158] and (b) those who do not qualify the relation of the epithelium with the intervillous space [9 141]
7 The intervillous space is lined by a layer of vascular endothelium and a layer of decidual cells [58]
8 The intervillous space is a sac lined by maternal blood vessel endothelium
9 The intervillous space consists of lacunae or sinuses lined by decidual cells [1 4 4]
10 The intervillous clefts are extravascular spaces —in the decidua—in which a kind of secretion (uterine milk) is found without any special course of circulation [69] those who believed the uterine milk to be mixed with blood [7] and those who believed in the uterine milk theory and also thought that there is no intervillous space [130]
11 The intervillous spaces are lymph spaces that contain lymph [85]
12 The intervillous space a cavernosus area is primarily of fetal origin (coalescing of trophoblastic lacunae) It is lined by trophoblastic tissue (fetal side) and deciduae (maternal side) [13 1 4] (The present accepted concept)

PRESENT CONCEPTS CONCERNING THE PROCESS OF IMPLANTATION

Nidation in the human until the recent work of Hertig and Rock [165 70 1 6] had remained an unexplained mystery Their discovery and description of a series of early implanting human embryos in conjunction with the earlier findings in the rhesus monkey [174] allow one to hypothesize a continuous process from fertilization through placentation

At the time of implantation at approximately six days following ovulation the human ovum has developed to the blastocyst stage beneath the embryonic disc Surrounding the blastocyst is a thin layer of trophoblastic cells The blastocyst loses its zona pellucida and as a result the adhesive qualities inherent in the trophoblast come into play At the site of contact with the endometrium the trophoblast undergoes proliferation forming both cyto and syncytial types The proliferating trophoblast erodes through enzyme activity into the endometrial glands and vessels forming many small lacunae which contain a mixture of blood and glandular secretion

The outward appearance of the earliest recorded human implanted embryo (7 5 days) [170 71] was that of a slightly raised oval area with a round opaque zone in its center The surrounding area had a chicken skin appearance due to the prominent gland openings on the endometrial surface (Fig 1)

At 9 5 days [1 0 1] the site of implantation is quite hyperemic with the area of the embryo identified by a raised oval papule on the endometrial surface The trophoblastic development is more pronounced at the embryonic pole than toward the aembryonic area The syncytiotrophoblast cells have increased numerically with a coalescence of many of the primitive lacunae having occurred On the other hand the cytotrophoblast cells line the chorionic cavity with evidence of early mesoblasts being formed These will eventually form into the connective tissue and vessels of the placenta [165]

At 11 12 days [17] the syncytiotrophoblast is thick containing numerous blood filled lacunae (Fig 2) Apparently some syncytiotrophoblasts are actively

As early as William Harvey[65] the opinion of the separateness of the maternal and fetal circulations was held Harvey believed that the umbilical vessels ended in filaments in the cotyledons where they came in contact with and imbibed a jelly or mucilage similar to albumen manufactured by the mother He supported his ideas by experiments on animals and through observations on the human claiming that at no time had he ever found blood in the placenta but instead a white jelly like material He came to the conclusion that the placenta acted as the nutriment body of the embryo Among his *observations he noted that the pulse of the fetus* was much faster than that of the mother a further indication that the two circulations were separate Some believed that the union of the uterus and placenta is effected by means of lymphatics which belong to the fetal circulation and are not directly connected to the maternal circulation[9] Though many agreed on the separation of the maternal and fetal circulations the vesicular pattern varied from that of fine hair sized blood vessels which took up a whitish substance from the uterine glands for nutriment of the fetus to those who thought the two circulations were capillary plexuses (maternal and fetal) in close apposition[45 19 13]

THE INTERVILLOUS SPACE CONTAINING BLOOD

Although Galen spoke of a space under the cotyledons Giulio Cesare Aranzio[10] a student of Vesalius was one of the first to describe a placento uterine space which has been variously termed cavernous space cells of the placenta placental sinus or lacuna placental bag or sac and intervillous space Vater[144] described the uterine vessels as opening into sinuses of the placenta into which villi *of the chorion dip like the roots of a plant to* absorb nutriment for the fetus He later repudiated his findings

MacKenzie a student of Smellie s procured the body of a pregnant woman who died undelivered at *full term* and injected with wax the maternal arteries and veins of the uterus with particular success John Hunter[73] acting in collaboration with MacKenzie found that the maternal arteries opened into a large space which was filled with a mixture of the colored waxes The veins appeared to open into this space and to have their colored contents intermingle with that of the arteries The area in which the injection mass was contained seemed a cellular structure fitted to be a reservoir for blood The blood according to Hunter is detached from the common circulation of the mother moves through the placental spaces and is then returned back into the course of the circulation of the mother

The researches of Weber[155] established firmly the presence of the intervillous space Weber used a method which employed inflation with a blowpipe slitting with scissors in order to follow the vessels into the intervillous space He states that the terminal uterine arteries enter the placenta without branching *the veins which by anastomosing with* each other frequently and at all points seem to form in this manner a system of cells or spaces from which the blood passes via the venous trunks into *the* uterine veins Reid[125] believed his injections and studies demonstrated the intraplacental part of the maternal vascular system to be dilated into a large sac with the maternal blood circulating through this sac Dalrymple[32] independently demon*strated the presence of the intervillous space* as had Weber and Reid

Goodsir[58] described the presence of sinuses in the placenta which appeared to be formed by maternal blood vessels Van der Kolk[143] described the interior of the placenta as being subdivided into compartments Numerous other workers following their own studies agreed in the existence of the intervillous space[4 2 3 4 49 94 67 30 1 9 127 5 141]

On the other hand another large school of workers believed that the blood of the mother is not carried into the interior of the placenta that the placenta is entirely a fetal organ hence the blood vessels of the uterus and placenta cannot intermingle and the intervillous space is nonexistent[1 9 121 13 1 5 1 103 74]

Another was of the opinion that the vessels of the chorion do not meet the mouths of the uterine vessels Instead the placental arteries and veins break up into finite capillaries which empty into the placento-uterine space and mix fetal blood with that of the mother thus resulting in an indirect blood communication between the fetus and the mother while others described the presence of sinuses in the placenta and felt that the two circulations (maternal and fetal) united[10 118 17]

Not until John Hunter s[73] observations on the in*jected placenta of MacKenzie was the theory* of the intervillous space as well as the concept of separate maternal and fetal circulations brought forward under one concept This work remained under a great deal of discussion until Weber[1 5] firmly established through his injection studies on the placenta that the intervillous space with separate maternal and fetal circulations exists This has become known as the Weber Doctrine and has since been the basis upon which the modern concept of the placenta has been built

Since Weber many observers with better techniques have confirmed the existence of the inter

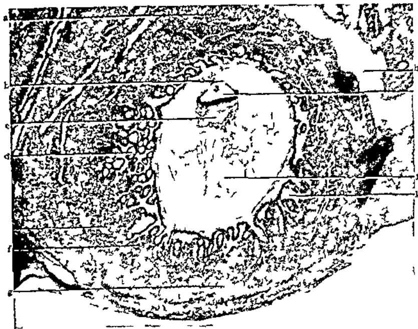


FIG 3 A SECTION THROUGH A 16 DAY-OLD HUMAN OVUM Observe the embryonic shield with the amniotic cavity (i) above it and the secondary yoke sac (*c*) below it The dark chorion encloses the large exocoelom (*f*) and is connected to the embryo by the mesodermal body stalk (*b*) Secondary villi containing cores of mesoderm and angioblasts are in process of development (*d*) Peripheral to these is a lamina composed largely of cytotrophoblast constituting cell columns (*f*) and the developing trophoblastic shell (*e*) Surrounding the latter with an indefinite boundary between them is the decidua Below separating the egg from the uterine cavity is a broad zone of decidua capsularis (*g*) *a* Decidua basalis *k* intervillous space *h* dilated maternal venous sinus × 30 (Courtesy of Drs Hertig and Rock from Greep Histology Blakiston Company)

eroding into blood vessels but where this is occurring the lacunae have not coalesced into the future intervillous space This according to Hertig and Rock[170] may be a check valve mechanism for protection against total displacement by hemorrhage The cytotrophoblast has become more prominent and forms an inner lining of the shell of the ovum Thickenings in the proliferating cytotrophoblast are pushing through the syncytiotrophoblast to form the beginning of villi The exocoelom of the embryo is spherical in shape with the outer chorionic cavity surrounding the embryo Lining the exocoelom is Heuser s membrane[173] beneath which are the primitive mesoblasts—the primitive connective tissue elements of the placenta

At 16 5 days[170] the ovum on inspection revealed an oval raised macular area slightly translucent with a finely mottled congested appearance The *Schluss coagulum*[170] is easily recognizable following fixation The gland openings are no longer prominent (Fig 3)

Early branching chorionic villi attached to the chorionic membrane are evident Vascular primordia are definitely present The cytotrophoblast at the tips of the villi show more progress in growth into the syncytiotrophoblast in forming a new trophoblast shell Hertig and Rock[1] feel that the cytotrophoblast through such a villus formation follows the syncytiotrophoblastic framework which formed earlier as a vacuolization of the solid syncytiotrophoblast

Implantation for general purposes is at an end and placentation becomes a reality The lacunae in becoming confluent have begun the development of the intervillous space The cytotrophoblast cords covered with a layer of syncytiotrophoblast begin now to take on the characteristics of the primary chorionic villi The cytotrophoblast differentiates into a core of connective tissue which forms into small blood vessels and blood elements This becomes apparent at about 18 days Small buds are noted on the cell columns of trophoblast which will form into secondary villi The growth of the vessels of the villi continues as well as the increase in the size and coalescing of the lacunae eventually to form what will become the mature placenta

The allantoic arteries and veins passing from the embryo through the body stalk into the chorion join with the small vessels of the main villi to form a true vascular connection between the embryo and the placenta A shift has taken place from the primitive relationship during implantation wherein the trophoblastic tissue acted as an intermediary for the embryo through a histotrophic state for nutrition to



Fig 1 A 7½ day human ovum implanted on a physiologically edematous 22-day secretory endometrium ×300 (Courtesy of Drs Hertig and Rock from Greep Histology Blakiston Company)

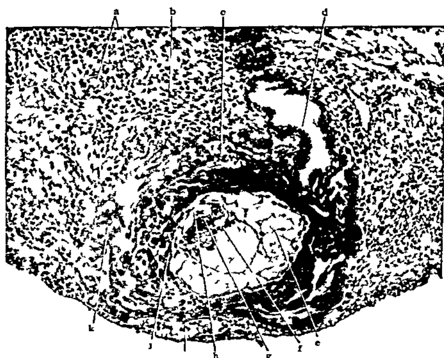


Fig 2 A section through an 11 day-old human ovum imbedded in a 25-day secretory endometrium Within the syncytial trophoblast (*k*) is an intercommunicative network of lacunar spaces which contain some extravasated maternal blood (*c*) The bilaminar embryonic germ disc is apparent in the center of the ovum with the amniotic cavity above and the primary yoke sac cavity below There is a suggestion of early decidua around the ovum Above the ovum to the right is an enlarged secretory endometrial gland (*d*) whereas above and to the left the edematous stroma contains a coiled artery (*a*) *e* Exocoelom *f* the centrally situated entoderm bounding the yoke sac *g* ectodermal embryonic shield *h* amniotic cavity enclosed by amnion which is delaminating *in situ* from the adjacent cytotrophoblast *i* repairing endometrial epithelium *j* cytotrophoblast giving rise to syncytium and extra embryonic mesoderm ×100 (Courtesy of Drs Hertig and Rock from Greep Histology Blakiston Company)

dergo a fusiform swelling not unlike a small sac from which small spiral vessels may exit They vary in numbers from one to as many as nineteen terminating in nozzles which directly enter the intervillous space These vessels correspond to the spiral arteries of the endometrium The small branches of the radials continue into the decidual plate and through a capillary plexus are continuous with the inner venous net No venous capillary anastomoses are apparent with the arterioles ending in the intervillous space For the most part this conforms with Okkels and Engle s classification of the endometrial arteries It is interesting to note that the diameters of these reserve sacs and the multiple terminal nozzles are apparently capable of extensive alterations in caliber (Fig 10) The nozzles may be as large as 1 mm in diameter with the sac 5 6 mm in size (Fig 11) On the other hand Bumm and Klein [3] basing most of their observations on post partum placentae describe the spiral arteries for the most part to be at the base of the septa between the cotyledons with a lesser number under the main area of attachment of the cotyledons The central area under the cotyledons according to Bumm is limited to the venous connections of the intervillous space It was his further impression that the differential between the arteries and veins could be made by the amount of blood and appearance of them

Subsequent workers after studying injected specimens have for the most part confirmed the arterial pattern as described by Spanner [50 40 87] (Fig 12)

Spanner states that the basalar endometrial arteries are connected through a capillary network with the inner venous network of the uterus (Figs 7 8) This network connects with the deeper venous system of the uterus with right and left sided anastomoses and leave the uterus through the large uterine veins (Fig 9)

Krantz [8] was able to demonstrate a venous pattern of the endometrium and myometrium similar to that reported by Spanner Three specimens were studied in the fifth sixth and seventh months of pregnancy (Figs 5 6 7 8)

To Bumm and his workers the venous drainage of the uterus was through veins that were situated under the central part of the cotyledons with an inconstant venous drainage at the periphery of the placenta Ramsey [1 3] points out that in early placentation venous connections are present These continue to decrease in number as the pregnancy progresses On the other hand this may be the case because the arterioles through their spiral structure are capable of accommodating the demands of the stretching endometrium and uterus as pregnancy progresses

The marginal sinus should be considered as a discrete entity from the superficial and occasional connecting veins underlying the placenta It can be defined as a peripheral portion of the intervillous space bounded by chorionic membrane and trophoblastic tissue bordered on one side by the intervillous space itself and on the other with the venous sinuses of the uterus Villi may be seen to occupy part of the marginal sinus

Spanner as well as Ramsey describes both the direct and indirect drainage of the marginal area (Figs 11 12 13A & B) The indirect is through veins which cross the trophoblastic plate in a zone approximately 1½ cm from the placental margin In the direct drainage the flow is through the endometrium and into the maternal veins Spanner believes that the marginal sinus area is a distinct entity a continuation of the subchorial space

Ramsey [1 3] considers the marginal sinus to be a group of blood lakes around the periphery of the placenta with or without villi and in direct continuity with the intervillous space The direction of



FIG 7 EXTERIOR VIEW OF INJECTED VENOUS MASS OF A PREGNANT UTERUS demonstrating the collecting veins—7½ months (*Krantz*)

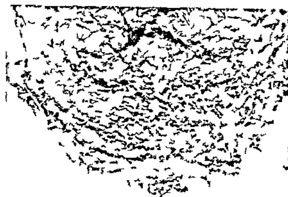


FIG 8 SUPERFICIAL VENOUS COLLECTING SYSTEM OF THE UTERUS—7½ months—with injected intervillous space and marginal sinus area (*Krantz*)

that of a circulating system between the embryo and that of the mother (intervillous space)—hemotrophic state of nutrition

PRESENT CONCEPTS CONCERNING THE UTERO PLACENTAL VASCULAR RELATIONSHIPS

To understand placental uterine relationships familiarity with the vascular pattern of the uterus is necessary. In reality placentation is the establishment of a working arrangement between the vascular systems of the host and the embryo conducive to the growth and survival of the embryo

During pregnancy the vascular pattern of the uterus changes rather markedly from that of the nonpregnant state. This is necessary in order to accommodate the increased blood supply to the area and changes in the size of the uterine wall and the uterus as a whole

The evidence at this time concerning the integral arterial supply (Fig 4) of the nonpregnant uterus according to Okkels and Engle[114] is as follows. The uterine ovarian anastomoses branch into the *myometrial arcuate arteries* and at the same time form cruciate anastomoses with the opposite side (Fig 5). The arcuates along their course give off branches at right angles called *radials*. These terminate in the spiral and basalar arterioles of the endometrium (Fig 6)



Fig 4 Main branches uterine artery pregnant uterus —5 months (Krantz)

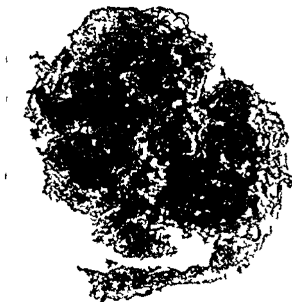


Fig 5 Injected arterial mass of pregnant uterus—5 months (Krantz)



Fig 6 Spiral arterioles pregnant uterus—5 months (Krantz)

Spanner[136] noted in his studies of the injected pregnant uterus a definite arterial pattern. This pattern for the most part is similar to that later described by Okkels and Engle[114]. Spanner describes an extensive anastomosis between the ovarian and uterine as well as those of the right and left sides of the uterus. He demonstrated branches of the uterine arteries that course parallel under the surface of the uterus (apparently arcuate in type) sending off direct perforating branches (radials) which penetrate through the extensive venous network terminating in two specific types of spiral arterioles. He noted that the penetrating (radial) arteries undergo several convolutions after passing through the inner venous net. At their termination these penetrating vessels un

venous drainage is therefore threefold—through the veins in the decidua under the cotyledons directly into the uterine veins at the periphery and through the marginal sinus (Fig 13)

The actual existence of the marginal sinus has been doubted by recent investigators Further injection studies as well as histologic sections will be necessary before this can be solved

STRUCTURE OF THE CHORION AND ITS VILLI

Covering of the Villi

The various ideas concerning the envelope about a villus can be summarized as follows

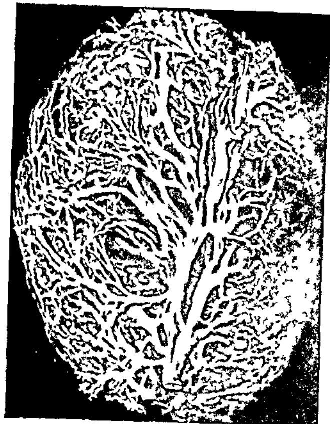
1 The epithelium of the villi is simple and of fetal origin It is a true epithelium [88 86]
2 It is simple and of maternal origin that is from uterine epithelium or endothelium and maternal connective tissue [142]
3 It is (in its later stages) of simple maternal connective tissue origin made of decidual cells [44]
4 The villus epithelium is of two layers of fetal origin The inner layer of connective tissue cells (layer of Langhans) and an outer ectodermal layer of epithelium [90]
5 It is of two layers The inner layer is fetal epithelium over which is endothelium of the dilated maternal vessels [158]
6 The villi are covered merely by maternal epithelium that is endothelium of the maternal blood vessels or at least a membrane continuous with it [125]
7 The epithelium of the villi is of two layers the inner layer is of maternal connective tissue decidual origin the outer layer is of maternal blood vessels endothelium as in Nos 5 and 6 [14 1 9 58]
8 It is of two layers the inner layer is epithelium of fetal origin the outer originating from maternal epithelium of the uterine gland into which the villi have grown [77]
9 It is of two layers both layers are of fetal and ectodermal (epithelial origin the outer layer being a syncytium and possessing a ciliary border [81 05 89 137]
10 It is of three layers a double fetal layer in contact with a maternal blood vessel endothelium [83]
11 It is of three layers all of maternal decidual cell origin [143]
12 The epithelium of the villi is made up of three layers which separate the maternal blood from the fetal They are *first* the walls of the fetal capillaries *second* a gelatinous membrane in which the vessels ramify and course and *third* an external nonvascular membrane derived from the chorion [49]
13 The epithelium of the villi is that of maternal blood vessels excepting sites into which the villi have eroded [8 151 88]
14 Weber spoke of a pellucid margin on the villus but did not recognize this membrane as being cellular (The cell theory came seven years later)
15 Dalrymple [3] was the first to recognize the nucleated nature of the border described by Weber He saw its resemblance to an epithelium



FIG 10 SIX MONTH UTERUS Corrosion preparation View of the placental site from the uterine cavity Observe the arterial spirals and their openings into the intervillous space (From Spanner R Mutterlicher und kindlicher kreislauf der menschlichen placenta und Seine Strombahnen Ztschr fur Anat & Gynec 71 351 1956)

It is no longer doubted that the work of Langhans [90] showing the presence of two layers of fetal origin is correct but he erred in his interpretation that the inner layer was composed of connective tissue cells it is now known that it also is of ectodermal origin Number 9 in the foregoing list is the *accepted interpretation*

Wislocki and Dempsey [160 161] employing electron microscopy studied placentae at ten weeks and at



(1)



(2)



(3)

FIG 9 (1) SEVEN MONTH UTERUS Corrosion preparation following venous injection (2) SEVEN MONTH UTERUS *Arteries and veins* (3) EIGHT MONTH UTERUS *Arterial spiral in the decidua emptying into the intervillous space* (From Spanner R Mutterlicher und kindlicher kreislauf der menschlichen placenta und Seine Strombahnen Ztschr fur Anat und Entwicklgesch 105 1799 1936)



(1)



(2)



(3)

Fig 12 Placental circulation maternal and fetal (1) Cross section of a pregnant uterus (39 weeks gestation) showing latex injection of vessel The placenta is attached to the posterior wall Blue latex fills the myometrial and uteroplacental veins the marginal zone vessels and areas of the lake under the fetal surface of the placenta Red latex is seen in the intervillous space in the central part of the placenta Scale in centimeters (2) Magnified portion of a cross section of the latex injected specimen shown in (1) placenta (above) partially dissected from uterus (below) to the left of center red latex is seen issuing from a terminal arteriole in the decidua and flowing into a cotyledon Also located in the decidua is a uteroplacental artery filled with red latex and cut in four segments Below the center of the field is a portion of a uteroplacental artery showing 21 convolutions Scale in millimeters (3) Shows a uteroplacental artery discharging its contents (red latex) into the intervillous space The terminal narrowing of the vessel as originally described by Spanner is well shown The spray streams out of the arterial mouth into the intervillous space A portion of an anchoring villus is present at the left Scale in millimeters (From Farn and Nicholson The Placental Circulation Maternal and Fetal Am Journ Obst & Gynec 63 1 5 1952)



(1)

(2)

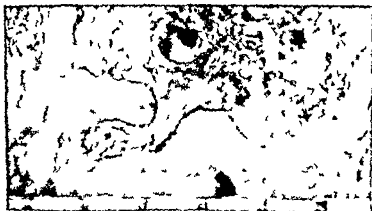
Fig 11 (1) Isolated uteroplacental arterial spirals obtained by injection and a corrosion preparation (2) Section through the marginal zone Note the size of the veins draining the intervillous space (From Spanner R *Mutterlicher und kindlicher kreislauf der menschlichen placenta und seine Strombahnen* Ztsch fur Anat & Gynec 71 351 1956)



(1)



(2)



(3)

FIG 12 PLACENTAL CIRCULATION maternal and fetal (1) Cross section of a pregnant uterus (39 weeks gestation) showing latex injection of vessels The placenta is attached to the posterior wall *Blue latex fills the myometrial and uteroplacental veins the marginal zone vessels and areas of the lake under the fetal surface of the placenta* Red latex is seen in the intervillous space in the central part of the *placenta Scale in centimeters* (2) Magnified portion of a cross section of the latex injected specimen shown in (1) placenta (above) partially dissected from uterus (below) to the left of center *red latex is seen issuing from a terminal arteriole* in the decidua and flowing into a cotyledon Also located in the decidua is a uteroplacental artery *filled* with red latex and cut in *four* segments Below the center of the field is a *portion* of a uteroplacental artery showing 24 *convolutions* Scale in *millimeters* (3) Shows a uteroplacental artery discharging its contents (red latex) into the intervillous space The terminal narrowing of the vessel as originally described by Spanner is well shown The spray streams out of the arterial mouth into the intervillous space *A portion of an anchoring villus* is present at the left Scale in millimeters (From Farn and Nicholson The Placental Circulation Maternal and Fetal Am Journ Obst & Gynec 63 1 5 1952)

term (Fig 14) Electron micrographs of the chorionic villi of the earlier specimen revealed abundant micro-villi on the surface of the syncytial trophoblast with numerous mitochondria and uniform sized vesicles within the interior of the cells (Fig 15) The vesicles are regarded as a form of ergastoplasm Other vacuoles more superficially seen in the syncytium are thought to be pinocytosed vacuoles of fluid

At term the chorionic villi are covered by syncytium (Fig 18) though frequently flattened basal residual cytotrophoblast (Langhans) cells are found Evidence is at hand that the Langhans cells are not separated from the syncytium or encapsulated by a basement membrane collagen or any other interstitial material (Figs 15 17) According to Wislocki and Dempsey *in the thinnest places in the* placental barrier where sinusoidal capillaries indent the syncytium there is a layer of syncytium a basement membrane (collagen) and a capillary wall with an endothelium present (Fig 16)

Stroma of the Villus

It was described by Farre [49 58] as a soft pulpy mass enveloping the blood vessels and as containing numerous cells Stieve [1 9] describes the stroma of the villi as being made of a matted connective tissue with collagen fibers coursing in all directions Between the fibers are numerous fibroblasts whose nuclei are long and cylindrical (2-3 μ thick and 12 15 μ long) Many of the nuclei are nodular The cytoplasm is small in amount and stretched out Many histiocytes (Hofbauer cells) are present and within this stroma are seen numerous arterioles venules and capillaries as well as mesenchymal tissue [15]

According to Wislocki [59] a network of fibers (reticulum) can be distinguished in the villi with special stains [17] Spivack [135] while searching for nerves in the placenta described a reticulum in the villi similar to that seen by Wislocki

Blood Vessels of the Villi

William Harvey [65] described the fetal vessels in the cotyledons as filaments which were connected with the umbilical vessels The arteries of the umbilical cord ramify upon the surface of the placenta sending branches into its substance These branches within the substance of the placenta subdivide until they become continuous with the veins Progressive confluence of these veins results in the formation of a single umbilical vein [73] (Figs 19 20)

Early studies on injected placental material evidenced entrance of an arteriole into one or more villi within each of which a loop was formed and the emergent limb thereof communicating with a vein [155 a]

On the other hand some workers felt that within each villus the arteriole is accompanied by a venule the artery becoming continuous with the vein at the blunt extremity of the villus [1 5] while others found that one umbilical vessel enters each villus forming a simple loop and passing out for the most part without dividing [58] Its branches therefore course separately or anastomose into a single trunk before emerging from the villus

In addition to one or more arteries and veins each villus contains numerous capillaries with the arterioles coursing through the midportion of the villus stem and dividing into its branches [49] The capillaries present various forms of arrangement in some parts *resembling Malpighian bodies and others the* arrangement of pulmonic capillaries The blood passes from the capillaries into the venules (which retrace the course through the villus tufts) The capillaries of the villi gradually disappear toward the end of pregnancy until the condition of the blood vessels within the villi form a simple capillary loop Many modifications occur in the form of the vascular loops within the villi They may be simple compound wavy or much contorted and in parts varicose

Grosser states that each villus contains a core of one or two arterioles and of one or two larger venules These communicate with a capillary net that lies immediately beneath the surface of the villus These arterioles of the villi are of the nature of end arteries [9] Wienbeck [57] describes capillaries in the syncytium of the villi Spanner [1] is in agreement with Grosser to the extent of finding a vascular core within the villus communicating with a subepithelial capillary plexus In addition he further describes a capillary plexus occurring in a comparable position in the villus stem He describes sphincter type valves in the medium and large size veins of the larger villi No valves were found in the placental arteries

Finn Bøe [19] states that the arteries of the chorionic plate divide into branches of relatively large size Three such divisions occur primary secondary and tertiary The latter give rise to an extensive paravascular plexus of vessels comprised of numerous short capillary size vessels with occasional arterio-venous shunts From this plexus arise the small short vascular units that constitute the fetal chorionic villi The distribution of the villi is equally numerous at the basal plate as well as near the chorion plate

On the other hand Spanner visualizes the fetal villi as forming an elaborate unit He describes the anchoring villus as passing from the chorion plate in an oblique manner to the basal plate where it turns and sends many branches into the intervillous

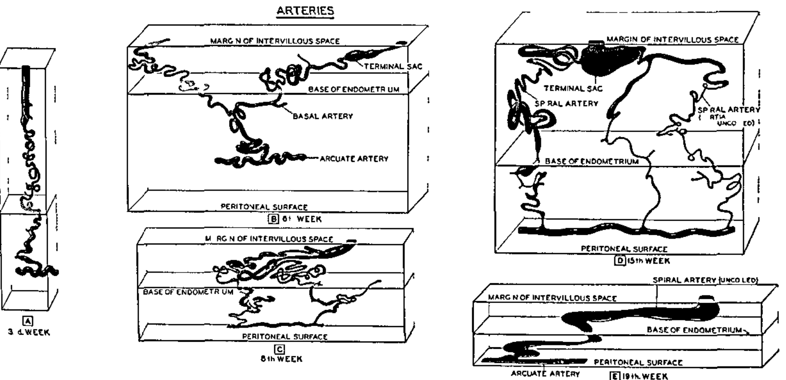


FIG 13A DIAGRAMMATIC REPRESENTATION OF THE COURSE AND CONFIGURATION OF THE ARTERIES OF THE MATERNAL PLACENTA OF THE MONKEY (*Macaca mulatta*) at various stages of pregnancy (From Ramsey E M The Vascular Pattern of the Endometrium of the Pregnant Rhesus Monkey Carnegie Institute of Washington Publication #603 Contributions to Embryology 35 151 73 1949) (From Ramsey E M 1956 Circulation in the Maternal Placenta of the Rhesus Monkey and Man with Observations on the Marginal Lakes Am J Anat 98 159 90)

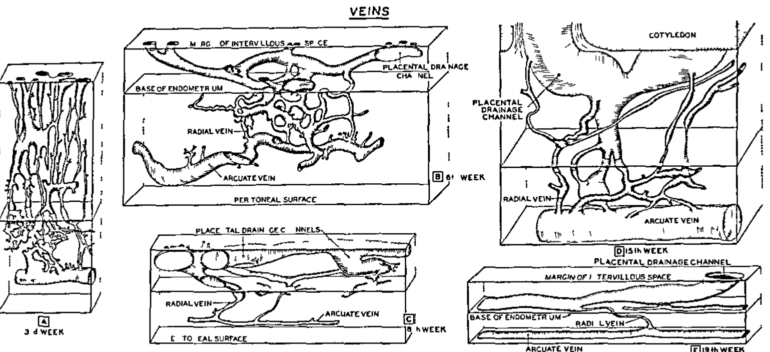


FIG 13B DIAGRAMMATIC REPRESENTATION OF THE COURSE AND CONFIGURATION OF THE VEINS OF THE MATERNAL PLACENTA OF THE MONKEY (*Macaca mulatta*) at various stages of pregnancy (From Ramsey E M The Vascular Pattern of the Endometrium of the Pregnant Rhesus Monkey Carnegie Institute of Washington Publication #603 Contributions to Embryology 35 151 73 1949) (From Ramsey E M 1956 Circulation in the Maternal Placenta of the Rhesus Monkey and Man with Observations on *the Marginal Lakes Am J Anat* 98 159 90)

space The picture is similar to that of an inverted willow tree This villus pattern has been commonly termed chandelier villus The main anchoring villi contain an artery and a vein surrounded by a capillary network A very extensive pattern of capillary plexus exists down to the terminal villi (Fig 21)

Lymphatics in the Villi

Many earlier workers believed lymphatic vessels to be present in the placenta and umbilical cord[155 16 70] Though they claimed such to be present no definite proof of their presence in the umbilical cord and placenta has been found[15 87 9] The so called lymph vessels may be areas of swelling and distortion due to fixation The most recent studies have shown an apparent absence of lymphatics[7 136]

Nerve Elements in the Fetal Placenta

A nerve originating from the coeliac ganglion and passing through the liver and accompanying the umbilical vein to the umbilicus and two nerves originating from the pelvic plexus and coursing with the paired umbilical arteries to the umbilicus were described by Schott[133] and confirmed by Gonner[55] A delicate system of nerve terminations in the chorionic villi was described by Fossati[51] the existence of which was doubted by Bucura though he and Gonner[55] did describe nervous elements coursing a short distance in the umbilical cord

Mabuchi[108] employing modifications of the Cajal and Bielschowsky methods claims to have demonstrated nerve fibers in the placenta and umbilical cord as early as the middle of the third month of pregnancy although they were sparse in number They were identified as nonmedullated supplying the blood vessels of the cord and villi where they terminated as free endings in the media Ganglion cells in the placenta or umbilical cord were demonstrated[102] During this same period other workers could find no evidence of nervous elements in the human placenta[155 64 61] Spivack[135] showed that the umbilical vessels of the cord proper in man are devoid of a nervous element A network found in the capillaries of the fetal membranes of man suggests the nervous terminal reticulum that has been referred to previously by other authors[106 51] Spivack did not think this network yielded definite enough evidence to place it with nervous tissue rather than with the reticulum of connective tissue Confirmation of Spivack s findings were made by Krantz and Arey[87]

Muscle in the Fetal Placenta

Happe[63] while studying the stroma of placental villi in specimens of the first month of pregnancy en

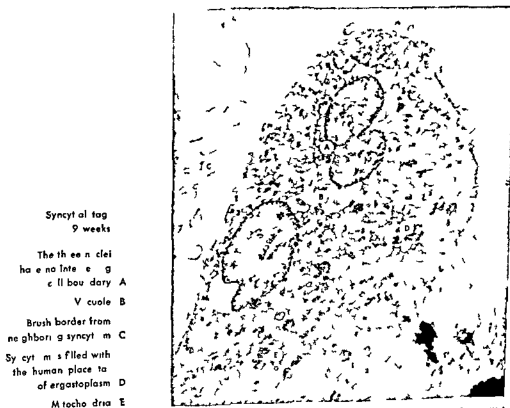


Fig 14 (Courtesy Dr E W Dempsey The Human Placenta What s New No 188 1955)

(1)

Syncyt um and cytot ophoblast
9 weeks

Langhans cells A

Nucleolus B

O moph l c l p d drop ets
conta n ng stero d horm nes C

M tochond ia D

These prof s spher of
structures co ta n ribon clear
p oteins (ergastoplasm) E

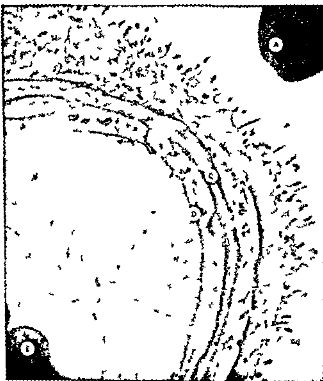
Process of p nocyto s
(t ansfe of plasma) F

Nucleated fetal red cells G

Irregula plasma membrane
between cytot ophoblast cells H

Fetal capillary wall I





(2)

T p of vill s at t m

Part of mater l r d cell A

B h b rd f y cytium B

Do bl l ye of f t l
co ect tissue w th
fib ls b tw C

E d thel m of fetal cap llary D

Pa t f f t l d c ll E

Fig. 15 (Courte y Dr E W De~psey The Human Placenta What s New No 188 1955)



FIG 16 SECTION THROUGH A THIN PORTION OF THE WALL OF A CHORIONIC VILLUS FROM A PLACENTA DELIVERED NORMALLY AT TERM The syncytial trophoblast has numerous slender microvilli which project into the intervillous space visible at the top of the figure Beneath the dark syncytium is an irregular pale zone containing a number of mitochondria and small granules This zone probably represents a thin cytoplasmic sheet of residual Langhans cell Beneath this zone are the basement membrane of the trophoblast and the endothelium which are separated by a connective tissue space Wisps of collagen can be seen as fibrils in this space Endothelium which rests on its basement membrane bounds the capillary lumen X 15 000 (From Wislocki B G and Dempsey E W Electron Microscopy of Human Placenta The Anatomical Record 123 165 1955)

countered some streaked elements which in structure appeared different than the connective tissue cells These elements possessed a characteristic longitudinal striation somewhat similar to that of smooth muscle fibers Streaked elements were dispersed in the connective tissue their longitudinal diameter was parallel to that of the villi He recognized the similarity of these elements to smooth muscle although he did not call these elements true muscle On the other hand Stieve [130] states that it is impossible to differentiate muscle in the villi of ova of four to six weeks and because of this Happe s streaked elements were probably endothelial cells of the early blood vessels

Iizuka [74] observed a layer of long spindle like cells with elliptical nuclei (which he calls smooth muscle) in the chorionic plate of the placenta These cells had a peculiar hue when stained by the Mallory method These spindle like cells formed a definite layer which was thicker in the central part of the placenta Iizuka could not explain the time of the muscle s appearance in the placenta during gestation the development of the muscle cells and the possible function of this muscle layer According to Sanger and Herff [131 83] part of the mesodermal tissue of the human amnion transforms into contractile elements (as has been demonstrated in birds) From this Naujoks [110] who cited these authors hypothesized that the amnion in the human placenta contracted It has been hypothesized that the smooth muscle in the amnion and chorion would help resist spontaneous rupture of the membranes during labor [110]

Dubreuil and Riviere [38] described what they identified as smooth muscle elements in the chorion plate and in the villus trunks The material used consisted mainly of full term placentae spontaneously delivered or extracted by cesearcean section One placenta however came from a fetus of 71 mm The specimens were fixed in 5 per cent formaldehyde embedded in paraffin or celloidin and stained with hematoxylin picro red hematoxylin pyrosine safran hematoxylin fuchsin aniline blue or by a method of Del Rio Hortega The muscle fibers were bright red with a violet nucleus while the protoplasm of the adjoining cells remained a pale pink The protoplasmic strands (cytoplasm of the muscle cells) are narrow and thread like they do not connect with the neighboring cells the nucleus is cigar shaped and ten to fifteen times as long as wide The muscle

compared favorably with that seen in the blood vessels Instead of grouping in bands with little connective tissue interspersed between them they have this peculiarity they are disseminated in a fibrous connective tissue which is likely to mask them to superficial examination The connective tissue is more abundant than the muscle tissue For this reason Dubreuil and Riviere called this whole formation in the human placenta formation fibro musculaires The connective tissue and the muscle fibers appear to occur in bundles which they described as follows

In the chorionic plate the fibromuscular formations were distributed parallel to the placental surface between the large vessels and the intervillous space They form a layer of varying thickness which may be completely absent especially in the areas where fibrin atrophy is present After leaving the chorionic plate the muscle bands penetrate the principal villus trunks of which they became the main framework The fibromuscular formations are easily distinguished from the muscular tunic of the blood vessels in the



Fig 17 Section through the wall of a villus showing profuse and elongated microvilli projecting into the intervillous space Several large vacuoles are present in the apical syncytium (arrows) The nuclei of two Langhans cells and a portion of a nucleus of the syncytium are visible × 8000 (From Wislocki G B and Dempsey E W Electron Microscopy of Human Placenta The Anatomical Record 123 153 1955)



Fig 18 1 Section through a portion of a Langhans cell and the overlying syncytium An edge of a nucleus (*N*) below The figure illustrates the differences between the mitochondria (*M*) of the two regions In addition a dark fat droplet and some dilated ergastoplasmic sacs appear in the syncytium × 15 000 2 Another section through a villus illustrating a variation in the appearance of the surface microvilli Mitochondria ergastoplasm and granular cytoplasm are also apparent × 8000 3 An area from the surface of the syncytium exhibiting several of the large apical vacuoles including one within a promontory which projects into the intervillous space × 16 000 (From Wislocki G B and Dempsey E W Electron Microscopy of Human Placenta The Anatomical Record 123 155 1955)

large villi These bundles course separately though run parallel to the muscular tunic of the blood vessels and exchange numerous fibers with other muscle fiber formations in the same villus In the smaller villi (those of medium size) there is usually one fibromuscular bundle Usually the venules and capillaries are forced to the periphery by this fibromuscular tissue this framework of fibromuscular band does not make contact with the syncytium In good sections the fibromuscular formations can be followed to the basal plate The earliest appearance of the fibromuscular formations in development was found to be in the placenta of fetuses as small as 71 mm Dubreuil and Riviere felt that the fibromuscular for

mations may play some role in the circulation of the maternal blood through the intervillous space

Guarna[61] repeating Dubreuil and Riviere's work on a large number of human placentae believed he found smooth muscle fibers to be present in the chorion and villi The sections were taken from various zones of the placenta from the fourth month through term Ova up to the third month were fixed totally

Guarna states that in younger embryos (those of a few weeks) the contractile elements do not appear as typical muscle fibers but as fusiform elements and that an exact differentiation cannot be made until the fourth month of pregnancy Even then the longitudinal striations are weak and stain much less markedly than those of the fifth month and far less than those in placentae at term He found the muscle to be present in the villi of large and medium sizes He confirmed Dubreuil and Riviere's findings of smooth muscle in the human placenta as to the position anastomoses and frequently of the fibers

Spanner[136] using both Heidenhain's iron hematoxylin and an eosin methylene blue stain reported smooth muscle in the chorion and main villus trunks of the human placenta He describes two types of smooth muscle in the chorion plate the spindle cell type and the shorter bandform type with processes extending from both poles which branch and whose terminations are lost in the connective tissue stroma Heidenhain's stain clearly differentiates the myofibrils while the eosin methylene blue differentiates the smooth muscle cells as a bright red on a blue background Contraction knots are noted in the cytoplasm of some of the fibers The spindle cells lie parallel to each other in groups or are singly stretched out the latter end to end The narrow nucleus is long (up to 35 μ) but may appear in other forms due to the angle of the section However it is usually situated within a vacuole in the muscle fiber Contorted nuclei are frequently seen even in those which were fixed first and then sectioned which is further evidence of the muscle fibers ability to contract

Steve and others were critical of all work done on the presence of smooth muscle in the chorion and villi of the human placenta excepting for fibers that belong to or are displaced from the blood vessels of the villi[139 60]

Krantz and Arey[87] employing specific stain for muscle (Milligan method)[104] described the presence of a significant amount of smooth muscle in the chorion plate and villi Two types of muscle fibers were described—the conventional spindle shape and the branching type (Fig 22)—the latter being quantitatively more numerous than the spindle type except in the chorion plate where both types appear in equal quantities In the small villi the spindle type predominates Favorable comparison can be made between these muscle types and other smooth muscle observed in the fetus and placental blood vessels

The smooth muscle elements as distributed in the chorion form a discontinuous layer of varying thickness parallel to the fetal surface of the placenta The fibers may be in contact with adjoining muscle cells or their terminations may end as free endings in the connective tissue stroma The muscle fibers in the chorion plate course predominantly in a circumferential direction more abundant in the area of insertion of the umbilical cord gradually becoming less numerous toward the periphery The circumferential fibers located in the periphery of the placenta are as numerous as those which course radially through the chorion plate (Figs 24 25)

The muscle elements leave the chorion plate and penetrate the principal villus trunks (anchoring villi) These trunks contain the most diffuse muscle which courses outside the vascular tunic entirely in the longitudinal axis of the villi (Fig 23) The diffuse muscle fibers of significant number coursing in longitudinal axis of the villi can be followed in a similar pattern into the various branches of the villi (Fig 26) The muscle is distinguishable in villi to about the precapillary level of blood vessels where thereafter only an occasional fiber can be observed (Fig 27)

The muscle pattern in the vessels consists of interlacing spirals similar to that described by Reynolds in the umbilical cord[1 8] The smooth muscle of the blood vessels has no apparent limiting membrane A transitional zone lies between the muscularis of the blood vessel and the muscle of the villi The muscle fibers of the villi and blood vessels are similar in type and structure

COURSE OF CIRCULATION THROUGH THE INTERVILLOUS SPACE

Farré and His School

Farre[49] was one of the first to formulate a theory of the course of circulation of the maternal blood through the intervillous space He held that the decidua marks the maternal boundary of the placenta this layer serves as a medium for veins and arteries passing from the uterus to the placenta Many valve like apertures present on the maternal surface of a delivered placenta were described by him to be orifices of veins that had been torn away from the uterus during the process of separation He maintained that the arteries run a short *tortuous* course before they terminate abruptly upon the inner surface of the placenta while the veins enter directly The veins are to be found opening into the



FIG 19 CORROSION SPECIMEN OF A FULL TERM HUMAN PLACENTA The fetal circulation viewed from the *maternal* side (Illustration from a scientific exhibit on the human placenta by members of the Dept of Obstetrics and Gynecology University of California School of Medicine)

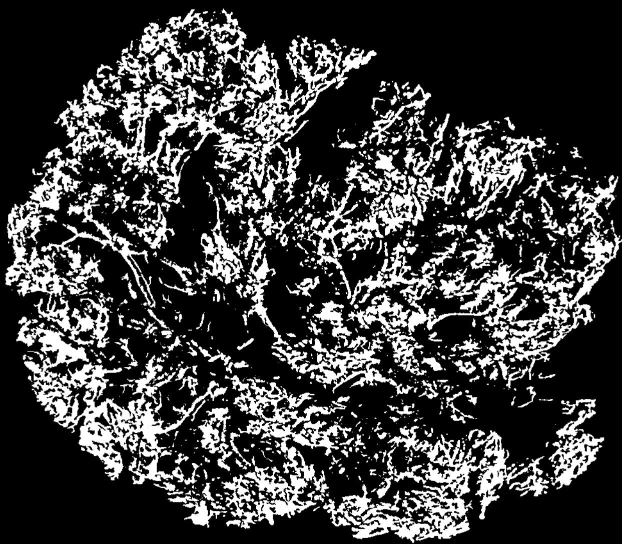


FIG. 20 CORROSION SPECIMEN OF A FULL TERM HUMAN PLACENTA The fetal circulation viewed from the fetal side (Illustrations from a scientific exhibit on the human placenta by members of the Dept of Obstetrics and Gynecology University of California School of Medicine)

intervillous space between the septa on the sides of the septa and into the circular sinus at the periphery of the placenta while the arteries enter the intervillous space between the septa He observed that



FIG 21 ANCHORING VILLUS DISSECTED FROM A TERM PLACENTA (*Krantz*)

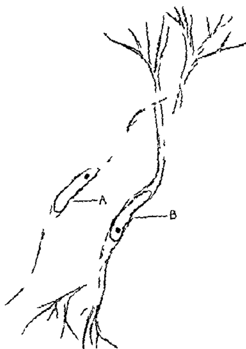


FIG 22 TWO TYPES OF MUSCLE FIBERS PRESENT IN THE HUMAN PLACENTA A spindle type B branching type (*Krant & Arey*)



FIG 23 SMOOTH MUSCLE IN A VILLUS It is diffuse and is wholly outside the blood vessel muscularis but blends into it (Krantz Obstetrics Greenhill 11th ed Saunders 1955)



FIG 24 SMOOTH MUSCLE IN CHORION PLATE (Krantz Obstetrics Greenhill 11th ed Saunders 1955)

the interspaces left between the villi were widest at the roots of the villi and smallest at their extremities Farre states that the maternal blood after having its impetus diminished by the spiral course of the arteries passes directly into the intervillous space where the blood becomes immediately separated by the villi into many fine streamlets Consequently the interior of the organ is broken up into countless channels After flowing among the villi the blood

leaves the intervillous space by the venous orifices located on the surface of the decidua upon the dissepiments and through the marginal furrows from which it is conducted to the venous sinuses in the uterus He believes that the chorion is responsible for confining the maternal blood within the placental channels also that the force and amount of the maternal blood increase as the organ grows in size during pregnancy As a result of the placenta being partitioned by septa the blood volume is divided among many smaller areas therefore reducing the danger of placental rupture

Bumm and His School

Bumm [3] formulated a theory of the circulation of the intervillous space based on his own observations and those of others of the same period [105, 111, 18, 153, 66, 9] He stated that venous openings into the intervillous space may be seen grossly in the mature postpartum placenta Furthermore he claimed that these veins neither branch nor ramify After injecting the veins with a colored gelatin he could trace the material

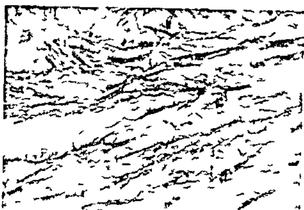


FIG 25 HIGH POWER PHOTOMICROGRAPH OF MUSCLE IN THE CHORION PLATE × 940 (*Krant*.)



FIG 26 SMOOTH MUSCLE FIBERS IN ANCHORING VILLUS (*Krant. & Arey*)



FIG 27 SMOOTH MUSCLE IN SMALL VILLUS—pattern runs in longitudinal axis of villus (*Krant & Arey*)

into the intervillous space His observations on fixed placentae were as follows

A VEINS

1 Venous Wall The luminal surface is covered with an endothelium It usually ends at the junction of the vein with the intervillous space It may however extend beyond the junction and into the intervillous space Surrounding the endothelium is a layer of connective tissue which varies in amount with the size of the vein As the vein approaches the intervillous space there is an intermingling of the decidual cells with the connective tissue elements The vein is composed of a mere endothelium surrounded by decidual cells at its point of junction with the intervillous space

2 Course The veins approach parallel to the placenta make a right angle turn and communicate with the intervillous space The caliber of the junctional orifice varies in size from mere slits to large round apertures Free villi may extend into the orifices of the veins The venous orifices are between the septa and usually in the center of the cotyledon

B ARTERIES

The arteries are smaller than the veins and may be empty Consequently they are harder to identify than the veins The arteries course in the septa on the border of the cotyledons The arteries follow a tortuous course and may be seen as many as 15 20 times within one section [3] One can trace them directly into the intervillous space If the intervillous space is injected with a colored lime compound one will find the material (if enough pressure is used) to have penetrated into the lumen of the arteries

1 Wall of the Arteries The lining is similar to that of the veins being composed of a simple layer of endothelium External to the endothelium is a

layer of connective tissue Dispersed among the connective tissue fibers are many cigar shaped nuclei which resemble those of muscle cells The walls of the arteries are thicker than those of the veins

2 Course The arteries pursue a tortuous but essentially straight course to the septa They open into the intervillous space at the base and sides of the septa According to Bumm [3] each cotyledon has its own individual circulation since the arterial blood enters at the septa and courses between the villi and finds an exit through the veins in the middle of the cotyledon It thus forms a completely independent vascular unit The exit of the blood from the peripheral cotyledons may be double one being the usual venous drainage and the other being the peripheral sinus if present

Spanner and His School

Spanner after studying the anatomy of the gravid uterus and placenta formulated a new theory of circulation of the intervillous space His conclusions based on placentae injected *in situ* and then corroded were contradictory to most of the previous theories and observations regarding the vascular supply and circulation of the placenta He states that cotyledons (except peripheral) have no direct venous drainage Peripheral cotyledons have some veins at the margin in addition to the marginal sinus He further states that the uterine venous system consists of two networks the first situated just below the decidua and a second deeper network which drains into the main tributaries of the uterine and ovarian veins From his carefully injected preparations he concluded that the uterine arteries become tortuous swollen and discharge through tiny nozzles directly into the intervillous space throughout the floor of the cotyledons and not at the base and sides of the septa as had been previously described by Bumm and others The fetal vessels according to Spanner are contained within large villus trunks that originate in the chorion plate and pass directly through the intervillous space into the maternal decidua where they turn back on their course to fill the intervillous space Within the intervillous space the villi with their many branches appear like an inverted weeping willow tree Some branches arise from the main trunk (anchoring villus) on its way to the basal plate Each cotyledon is composed of a villus trunk (anchoring villus) near its middle which passes from the chorion plate to the basal plate (maternal decidua) spreading out in the maternal decidua to return upward as free floating branches (chandelier villi) to fill the greater part of the space between the chorion plate and the maternal decidua The number of large branches given off the main trunk during its passage from the chorion plate to the maternal decidua may vary These branches intimately entwine with those belonging to the chandelier villi An area about 5 10 mm in thickness devoid of villi exists between the chorion and the intervillous space This area is called the subchorionic blood lake and is not apparent in a collapsed detached placenta In his opinion the septa form complete boundaries to each cotyledon at the maternal surface (decidual surface) but fall short of reaching the chorion plate According to his findings the branches of the villi do not transgress beyond the septa and from this he concluded that each cotyledon forms a fetal circulatory unit but not a maternal one The maternal blood returns to the maternal veins of the uterus by flowing up through the intervillous space past the villi and into the large subchorionic lake The blood within this lake flows over the septa toward the periphery of the placenta where it pours into the orifices of the peripheral sinus and from the peripheral sinus into the superficial and deep uterine venous plexus The peripheral sinus is composed of numerous small and large communicating sinuses and may or may not completely surround the entire placenta The number of cotyledons according to Spanner varies from 14 to 30 in individual placentae

Stieve [139] did not agree with Spanner and in 1940 described his researches He concluded (1) venous outlets are to be found in all parts of the placenta and not as Spanner had previously stated (2) in the veins through which the blood leaves the intervillous space there are special adaptations in the form of valves and muscle cuffs which regulate the blood stream in its course toward the maternal heart (3) Spanner s statement that there is no fusion of the septa with the chorion plate is incorrect and (4) the careful researches of Bumm should not be hastily discarded for they are still in greater part correct In rebuttal to Stieve s criticism Spanner stated that one cannot study the placenta sufficiently by microscopic sections only [6] He states that in microscopic sections the areas described by Stieve as veins are merely engorged arteries on their tortuous course through the decidua to the intervillous space where they terminate in many small nozzles He further states that one not only must do injections and corrosions to get the correct anatomical relationships but also these must be done on placentae that are *still in situ* if the normal relations are to be maintained and true interpretations are to be drawn

Ramsey and Her School

Ramsey [123] feels that there is considerable doubt as to the validity of Spanner s theory as to the circulation of the intervillous space As a result of her own researches she feels that even in face of the

anatomical differences in the studies the separation of arterial and venous blood in the intervillous space is effected by the villi in a similar fashion to a muddy stream entering a marsh The admixture in such instance is slow to occur due to irregular space clumps of mud and grass which deflect the flow She further states that the maternal viz a tergo is responsible as the driving force behind the arterial streams This force exceeds the pressure in the intervillous space by 60 70 mm of mercury and may be augmented by the constriction of the spiral arterioles The exit of flow is mainly in the direction of the subchorial lake and hence into the venous exits Part of the blood though slowly does find exit through the basal plate and marginal sinus The flow of blood is maintained through the veins in the uterus by the myometrial contractions

MECHANISM OF CIRCULATION THROUGH THE INTERVILLOUS SPACE

Maternal Agencies

The agency most responsible for the circulation through the intervillous space is felt by many workers to be the maternal blood pressure (viz a tergo) which forces the subchorionic lake blood into the veins [136, 47, 8, 71] Some workers consider the viz a *tergo to be equal in importance with the action of* the uterine musculature [81, 137]

Certain workers such as Grosser and Herff have held the uterine musculature to be the responsible agency [60, 65] It was their opinion that the maternal heart played no role in the circulation of the intervillous space Deloré [36] summed up the question of the propulsive force as being unsettled but thought that the insensible contractions of the uterus as described by Hicks may be responsible [69] However he felt that because they were so inadequately studied no definite conclusions could be made It has been shown through studies that the carbonic acid levels in the blood could control such contractions of the uterus This he believed may be the method by which the placenta and uterus solve the problem of circulation that is contractions of the uterine muscle resulting from such chemical irritation would cause reduction in the blood volume of the intervillous space

Several workers [15, 140] believe that the maternal blood in part leaves the intervillous space after each uterine contraction has finished because the force of the contraction on the venous sinuses diminishes their lumens sufficiently to reduce the volume which they can adequately handle however the uterine contractions alone are too infrequent to account for adequate circulation of the intervillous space On the other hand Pankow [116] believes that the uterine contractions could be of small enough magnitude not to be recorded by the usual methods yet great enough to play a significant role in the circulation of the intervillous space Spanner [136] and Schroeder [134] believed that the uterine musculature acts like a large blood vessel continually expanding and contracting The thought that the uterine contractions are controlled by idiopathic reflexes was proposed by Meyer Ruegg [101]

Falkiner [47] summarizes the action of the uterine musculature on the circulation of the intervillous space as follows (1) by altering the inflow of blood (2) by pressure on the blood lake in the placenta and (3) by altering the pressure in the venous outflow

Fetal Agencies

The circulation through the intervillous space is partly governed by villus pulsations [140, 15, 102, 71, 116, 101, 134, 136] These pulsations result when each small villus becomes erect and then relaxes with each systole and diastole of the fetal heart Falkiner [47] finds it hard to imagine that the rhythmic pulsations of the villi could be responsible for the circulation of the intervillous space Kearns states On the fetal side of the placenta the fetal blood pressure and heart action are the most important agents in the circulation of the intervillous space [8]

Dubreuil and Riviere were of the opinion that the fibromuscular formations described by them in the placenta could play a role in the passage of maternal blood through the intervillous space [35] They cite work previously done which showed that the *smooth muscle fibers in the spleen are responsible* for its ability to contract and expel the blood This Dubreuil and Riviere feel, could happen in the placenta that is the contraction of the fibromuscular formations could cause closer approximation of the chorion plate with the basal plate of the placenta resulting in a reduced capacity in the intervillous space the end result being an increase in the flow of blood from the intervillous space into the venous sinuses of the uterus This increased pressure would also aid the venous blood flow toward the umbilical vein Such a mechanism according to Dubreuil and Riviere would favor the evacuation of the intervillous space in a way much more effective than the uterine contractions However they found it to be impossible for them to ascertain the frequency and cause of contraction of the fibromuscular formations The theory of structure of the placenta upon which this theory was based is as that of Bumm which considers each cotyledon a separate clearly *defined circulatory unit* with veins and arteries *under each septal* defined cotyledon as previously discussed

In addition to the role given to the fibromuscular formations by Dubreuil and Riviere Guarna states that the fibromuscular formations help to regulate the intravillous circulation by assisting in the action of the fetal vessel musculature and thus facilitates the circulation of the intervillous space by creating the pulsations of the villi [61]

Maternal and Fetal Agencies

According to Stoeckel [140] the circulation of the intervillous space is governed by the maternal heart villus pulsations and the contractions of the uterine musculature That villus pulsations play an auxiliary role to the maternal blood pressure in the circulation of the intervillous space was felt by other observers [152]

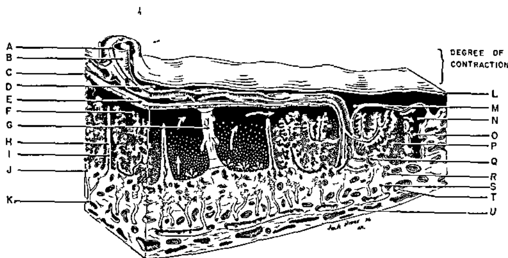
Spanner and Falkiner believe that the maternal blood pressure is the most important factor with the uterine contractions and the villus pulsations acting as accessory factors in producing a circulation through the intervillous space [136 47]

Kearns [82] believes that the maternal blood pressure in conjunction with the uterine contractions especially those of the internal longitudinal layer acts as a pumping mechanism on the maternal side of the placenta forcing the blood through the intervillous space On the fetal side the fetal blood pressure and the heart action are the most important agents Added to this there is a mechanical force the hydrostatic pressure which increases during uterine contractions This increased surface pressure on the thin walled subamniotic vessels mechanically supports the onward flow (of blood through the intervillous space)

Though Dubreuil and Riviere [3] felt the fibromuscular formations play a dominant role Guarna [61] held that the fibromuscular formations play an auxiliary role to the villous pulsations and the uterine contractions in the circulation of the intervillous space

The exact mechanism and course of flow of maternal blood through the intervillous space is still a point of conjecture Krantz [8] proposed the thought that the muscle in the human placenta occurs in sufficient quantity to warrant the placenta to be termed a peripheral heart He bases this statement on several factors (1) the apparent anchoring to the decidua of the central or anchoring villus of the cotyledons (2) the quantity of muscle present within these villi and (3) the direction of the muscle which is in the longitudinal axis of the villus The muscle

A UMBILICAL ARTERY
B UMBILICAL VEIN
C FIBRINOID DEPOSIT
D CHORION PLATE
E MUSCLE IN CHORION PLATE
F SUBCHORIAL SPACE
G MUSCLE TO VILLUS
H DECIDUAL SEPTA
I BASALAR ARTERIOLE
J DECIDUA
K ARCUATE ARTERY
L AMNION
M ANCHORING VILLUS
N MARGINAL SINUS
O UTERINE VEIN SUPERFICIAL
P INTERVILLOUS SPACE
Q SPIRAL ART WITH SAC—NOZZLES
R DECIDUAL VENULE
S RADIAL ARTERY
T UTERINE VEIN DEEP
U MYOMETRIUM



PLACENTA NORMALLY IMPLANTED
(BY KRANTZ modified after SPANNER)

Fig 28

upon contraction therefore would allow closer apposition to occur between the chorion plate and decidua This in turn would reduce the volume of the intervillous space The direction of flow (whether Spanner s concepts or those of Ramsey are adopted) might be as follows

The main route would originate at the nozzles of the spiral arteries with the greater flow passing amongst the villi of the intervillous space into the subchorial lake On contraction of the anchoring villus the blood would tend to flow into the collecting venous apertures and the marginal sinus at the periphery of the placenta The maternal blood pressure or viz a tergo would play a substantial role in forcing the blood upward in the intervillous space and aid in the circulation amongst the villi (Fig 28) The role of the inconstant nonrhythmic contractions of the uterus is dubious especially when one considers the placental arrangement in the uterus the directions of force from contractions and its possible role in such cases as abnormal implantation i e placenta praevia

As to the fetal circulation the muscle on contraction results in shortening of the villi This may be the responsible agent for maintaining the high venous pressure in the umbilical vein It therefore would assist the flow of blood through the villi by its milking action which is evidenced by the blood pressure in the umbilical vein (30 40 mm of mercury) being approximately one half of that in the umbilical arteries [1,6] What role the milking action of the villi may play in direct circulation and metabolite change in the intervillous space remains to be seen

The mechanisms causing the contraction of the smooth muscle are unknown However the following mechanisms can be considered (1) alteration of the pH by varying carbon dioxide tensions with its effect on smooth muscle action (2) anoxemia causing contraction of smooth muscle fibers (3) stretch response resulting from distention of the intervillous space (4) metabolites in the maternal blood which reach a level above that allowing normal exchange between the mother and fetus to occur and (5) hormonal control Nervous tissue has been conclusively demonstrated to be absent

In summary the following factors may be responsible for the circulation of the intervillous space 1 Maternal side (a) blood pressure and (b) uterine contractions 2 Fetal side (a) villus pulsations through fetal heart action and pulse (b) milking action of the longitudinal smooth muscle of the villi and (c) evacuation of the intervillous space by closer approximation of the maternal and fetal surfaces as a result of the contraction of the smooth muscle in the villi (peripheral heart)

ENDOCRINOLOGY

The pars distalis of the hypophysis has long been considered as the only organ capable of key hormone production However in recent years the human placenta has been found to be capable of many activities identifiable with the pituitary as well as with organs receiving pituitary stimulus

Since the work of Allen and Doisy and Frank and his co workers a great deal of productive research has been carried out concerning the relationship of the endocrines to reproduction [4,52,53] It was actually Aschheim and Zondek [11] who first made the significant observation of urinary excretion in varying amounts of estrogenic and gonadotropic substances in pregnancy Corner after isolating progesterone soon in conjunction with Allen demonstrated *the necessity of progesterone for the maintenance of pregnancy* Following isolation of crystalline estrogen [37] and progesterone [6] Browne and Venning [146] published their method of estimating progesterone excretion by means of pregnanediol determinations As a result of these fundamental studies the door to the endocrinological aspects of reproduction and the placenta was set again for further elucidation

Chorionic Gonadotropic Hormone

In 1927 Aschheim and Zondek [11] demonstrated the presence of a hormone complex in the urine This later proved to be estrogenic as well as gonadotropic substance Upon the latter is based the well known tests for pregnancy Many workers have conclusively demonstrated production of gonadotropin by the cytotrophoblast cells in tissue culture [80] Cytotrophoblast cells have been shown to persist beneath the syntrophoblast to term [161]

The structure of the chorionic gonadotropin (HCG) is apparently a glycoprotein with a molecular weight of approximately 80 000 It is bioassayed on the basis of international units which has been determined as 0 1 mg which is kept in a standard prepared dried form by the National Institute of Health London England This is approximately equal to one rat unit Serum and urine chorionic gonadotropin levels have been shown to be detectable in assayable amounts at approximately the 26th day of gestation and reach the maximum of production between the 60th and 70th day of pregnancy Thereafter the titer begins to fall until approximately about the 130th day when it reaches a level not too much in excess of that when it was first assayed (3000-4000 —to 4000 5000 international units) Following parturition the chorionic gonadotropin disappears in detectable amounts from the urine and serum within

a period of four days The function of the chorionic gonadotropin in human pregnancy appears to be the stimulation and maintenance of the corpus luteum for continued production of progesterone and estrogen Should these be allowed to lapse collapse of the endometrium would occur Therefore indirectly it maintains the endometrium as a suitable site for nidation and placentation Through diminished output a parallelism between chorionic gonadotropin production and blighted ova has been noted [84] In the nonpregnant human as well as in the monkey chorionic gonadotropin causes degenerative changes instead of the expected follicular stimulation and luteinization when administered during the follicular phase [43] The administration of HCG during the luteal phase of monkeys and women effects a prolongation of the functional aspects of the corpus luteum the progestin (progesterone and the 20α and βols of Zander) levels are maintained and menstruation is delayed for about fifteen days Though chorionic gonadotropin has many similarities to the pituitary gonadotropin it is essentially most probably structurally and physiologically an entirely different substance

Estrogen and Progesterone

In 1937 Browne and Venning [146] published their method of estimating progesterone excretion by means of determination of pregnanediol levels in both the urine and serum In addition to the previous work it has been shown that the excretion of pregnanediol increases progressively during the entire length of pregnancy reaching a maximum at approximately 28 weeks (200 days) [35] At this time the progesterone level drops off becoming negligible at the onset of labor [96] It was found that the removal of the ovaries after the first trimester did not appreciably influence the pregnanediol excretion in regard to the maintenance of pregnancy following the first trimester [120 1 29]

Progesterone has been isolated in a crystalline

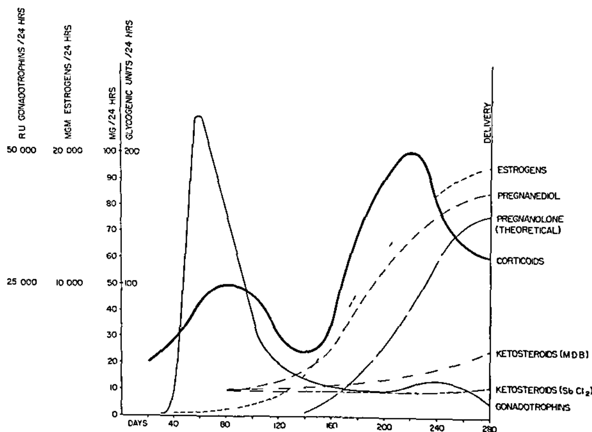


Fig 29 (*After Venning*)

form from the human placenta[118] It has been the impression of most investigators that the source of this hormone is from the syntrophoblasts (syncytium)

Estrogen

Estrogen was originally isolated from the urine with the gonadotropic hormone in pregnancy There has been a great deal of conjecture as to its function and source It has been well established that estrogen as well as gonadotropin is present in the urine blood and placenta with the placenta acting as the chief source[11 53 5 21 163 119] It has been noted that bilateral oophorectomy in the first trimester of pregnancy did not appreciably alter the excretion of estrogen in the urine[8 8 154] The site of production of estrogen within the placenta is most probably the syntrophoblast though it has not been conclusively demonstrated to be so by all workers The excretion of estrogen during a pregnancy progressively rises from the third month to its highest peak just prior to parturition Analysis of term placentae shows an extremely high level of estrogen whereas the ovaries at this time of gestation contain little estrogen The exact role of the balance between estrogen and progesterone during pregnancy is still a moot question (Fig 29)

Other Hormones

The apparent beneficial effects of pregnancy in causing a remission in a patient with Addisons disease has been demonstrated[76] Assali and Hamermeszs work on the apparent ACTH activity in dried chorionic villus tissue in conjunction with that of Gemzell who demonstrated values of blood levels of the 17 hydroxycorticoid steroids in pregnancy leads one to believe that the placenta produces ACTH (a chorionic adrenocorticotropic hormone)[13 56] Gaunt[54] in reviewing aldosterone pointed out that previous workers have demonstrated conclusively the presence of aldosterone within the human placenta[148 59 99 149] Its exact role is yet unknown Relaxin a hormone discovered by Hisaw has recently been isolated from placental tissue

BIBLIOGRAPHY

1 Adams F 1845 On the nature and treatment of floodings after delivery London Med Gazette New Series 1 755 8
2 Ahlfeld F 1883 Berichte und Arbeiten aus der Geburtshulflichen Klinik zu Giessen Leipzig (cited by Ruge in 1886)
3 Allan H and Dobbs E C 1935 Hormones in urine following oophorectomy during pregnancy Biochem J 29 285 7
4 Allen E and Doisy E A 1923 An ovarian hormone J A M A 81 819 21
5 Allen E Pratt J P and Doisy E A 1925 The ovarian follicular hormone its distribution in human genital tissues J A M A 85 399-405
6 Allen W 1932 The preparation of purified progestin J Biol Chem 98 591 605
7 Allen W and Corner G W 1930 Physiology of corpus luteum maintenance of pregnancy in rabbit after very early castration by corpus luteum extracts Proc Soc Exper Biol & Med 27 403 5
8 Amati C 1928 Placenta und ovarial hormon Zentralbl f Gynak 52 2639-45
9 Antoine T 1928 Über die Kotyledon und Septen der Plazenta Ztschr fur Geburtshilfe und Gynak 92 110 16
10 Aranzio Giulio Cesare (Arantius Aranzi) De Humano Foetu Opusculum Bologna (Translated to the French by Plozzoni 1564)
11 Aschheim S and Zondek B 1927 Hypophysenvorderlappenhormon und Ovarialhormon im Harn von Schwangeren Klin Wchnschr 6 1322
12 Asdell S A 1928 Growth and function of corpus luteum Physiol Rev 8 313-45
13 Assali N S and Hamermesz J 1954 Adrenocorticotrophic substances from human placenta Endocrinol 55 561 7
14 Bartelmez G W 1931 The contents of the intervillous space in the human placenta Anat Record 48 (Suppl) 9
15 Bischoff T W 1842 Entwicklungsgeschichte der Saugetiere und des Menschen Bd VIII des v Sommerringschen Handbuches Leipzig
16 Bloch R Beitrage zur pathologischen Anatomie und allgemeinen Pathologie herausgegeben von Prof Dr E Zeigler 1889 Bd Iv cited from Bumm E 1890 Arch f Gynak 37 1 15
17 Bloxam W 1839 On the structure of the human placenta and its connection with the uterus London Med Gazette New Series 2 74 5
18 Bøe F 1953 Studies on the vascularization of the human placenta Acta Obst et Gynec Scandinav 32 Suppl 5 1 92
19 Bonami M (1880s) Cited from Cazeaux F and S Tarnier Theory and Practice of Obstetrics 8th Am Ed pp 191 210 (1886)
20 Bonnet R 1903 Über Syncytium Plasmodian und Symplasma in der Placenta der Saugetiere und der Menschen Monatschr f Geb u Gynec 18 409
21 Browne J S L 1932 The chemical and physiological properties of crystalline oestrogenic hormones Canad J Research 8 180 97
22 Bucura C 1907 Über Nerven in der Nebelschnur und in der Placenta Zentralbl f Gynak XXXI Nr 48 p 1505
——— 1906 Ancora su di un caratteristico reticolo nei villi della placenta Ann di ost e g in Anno XXVIII 10 7
23 Bumm E 1889 Über uteroplacentar Gefasse Arch f Gynak Bd 35 520 ibid 1890 Zur Kenntnis der Uteroplacentargefasse Arch f Gynak 37 1 15 ibid 1893 Über die Entwicklung des mutterlichen Placenta Arch f Gynak Bd 43 192
24 Cheselden W 1712 The Anatomy of the Human Body Bowyer London 336 pp
25 Colucci G 1886 D alcuni nuovi dai di Struttura della Placenta Umana Napoli Cited by Waldeyer Arch fur Mikr Anat 35 1 51
26 Columbus Mattso Realdo 1559 De re Anatomica libri XV Venetus N Bevilacgoe pp 269
27 Corner G W and Allen W M 1929 Physiology of the corpus luteum II Production of a special uterine reaction (progestational proliferation) by

extracts of the corpus luteum Am J Physiol 88 326 39
28 Coste P 1842 Sur la formation de la caduque dans l oeuf humain Compt rend t 15 37 59 162 224
29 Caverly C F 1931 Ovarian cyst complicating pregnancy Am J Obst & Gynec 21 566 74
30 Cowper W 1698 The Anatomy of Humane Bodies by Rev C B Albinus Publ B Wolford Oxford London 54 8
31 Crawford J M 1956 The fetal placental circulation Part II the gross anatomy J Obst & Gynaec Brit Emp 63 87 90
32 Dalrymple J 1842 On the structure and functions of the human placenta Med Chir Soc Trans 25 21 9
33 Davenport H A and Kline C 1938 Staining paraffin sections with protargarol Stain Tech 13 147 60
34 Delfs E 1941 An assay method for human chorionic gonadotrophin Endocrinol 28 196 202
35 Delfs E and Jones G E S 1948 Endocrine patterns in abortion Obst & Gynec Survey 3 680 92
36 Delore X Recherches sur la circulation maternelle du placenta J de Med Paris 2 s 9 393 8
37 Doisy E A Veller C D and Thayer S H 1930 Preparation of crystalline ovarian hormone from urine of pregnant women J Biol Chem 86 499 509
38 Dubreuil G and Riviere M 1932 Formations fibromusculaires du chorion et villosites du placenta humain C R Soc Biol (Paris) 111 170 72 ibid 1933 Les formations fibro musculaires du placenta humain Quel rôle peuvent elles jouer dan la circulation placentaire Gynec et Obst 27 385-92
39 Duncan J M 1872 Note of a proof of the free intercommunication near the chorionic surface between the different parts of the system of maternal cells or blood-caverns of the placenta in the same and in different cotyledons Edinburgh Obstet J November 601 5
40 Earn N and Nichol on 1952 The placental circulation maternal and fetal Am J Obst & Gynec 63 1 5
41 Engle E T 1939 Gonadotrophic substances of blood urine and other body fluids In Allen Danforth and Doisy Sex and Internal Secretions 2nd ed Williams & Wilkins Baltimore p 1003
42 —— 1929 Ovarian responses Differences elicited by treatment with urine from pregnant women and by freshly implanted anterior lobe J A M A 93 276-7
43 —— 1932 Experimentally induced descent of the testis in the macacus monkey by hormones from the anterior pituitary and pregnancy urine Endocrinol 16 513 20
44 Ercolani G 1877 Sullunità del tipo anatomico della placenta nei mammiferi e nell umana specie e sullunita fisiologica della nutrizono dei feti in tutti i vertebrati Mem dell Accad di Bologna Ser *III* T VII fas 2
45 Eschricht D 1837 De organis quae respirationi et nutritioni foetus mamalium inserviunt—Hafnial Cited by Waldeyer 1890 Placenta Bau Meuschen and Offen Arch fur Mikr Anat 35 1 51
46 Fabricius ab Aquependente Hieronymus De formato foetu Francofurti imp J de Zetter 1600
47 Falkiner N 1939 Circulation of maternal blood through the placenta Irish Journal Medical Science 6 59 65
48 Fallopius G 1562 Observationes anatomicae Parisus apud B Turnsanium
49 Farre A 1859 The Cyclopaedia of Anatomy and Physiology R Todd Longman Brown Green Longman and Roberts London pp 545-725
50 Flourens M 1836 Recherches sur les communications vasculaires entre la mere et le foetus Ann Sc Nat Zool Ser 2 TV 65-8 or Comptes Rendus II 170-72 (1836)
51 Fossati G 1905 Sulla esistenza di un reticulo nervosa nei villa della Placenta Annali di Ost e Gin 27 402 ibid 1908 Über Nerven in der Nebelschnur und in der Placenta Zentralbl f Gynak 32 183-4
52 Frank R T Bonham D and Gustavson R G 1925 A new method of assaying the potency of the female sex hormone based upon its effect on the spontaneous contraction of the uterus of the white rat Am J Physiol 74 395
53 Frank R T Frank W L Gustavson R C and Weyerts W W 1925 Demonstrations of the female sex hormone in the circulating blood J A M A 85 510
54 Gaunt R Renzi A A and Chart J J 1955 Aldosterone—a review J Clin Endocrin & Metab 15 621-46
55 Gonner A 1906 Über Nerven und ernahrende Gefasse im Nebelstrang Monastchr f Geb u Gyn 24 453 6
56 Genzell C A 1953 Blood levels of 17 hydroxy corticosteroids in normal pregnancy J Clin Endocrin & Metab 13 898-902
57 Gey G O Seegar C E and Hellman L M 1938 The production of a gonadotrophic substance (prolan) by placental cells in tissue culture Science 88 306 7
58 Goodsir J 1845 Anatomical and Pathological Observations Edinburgh Nyles Macphail pp 50 63
59 Gordon E S Chart J J Hagedorn D and Shipley F G 1954 Mechanism of sodium retention in pre-eclamptic toxemia Obst & Gynec 4 39-50
60 Grosser O 1927 Fruhentwicklung Eihautbildung und Placentation Munchen J F Bergmann Publisher pp 88 89 170 340 41 345 381 2 395 400
61 Guarna A 1934 Sulla presenza di fibrocellule musculari liscie e di terminazioni nervose nella placenta umana e sul meccanismo della circolazione intervillosa Ann di Ostet e Ginecol 56 1231-44
62 Haller A 1854 Elementa Physiologiae Corporis Humani Lusaunae sumptibus M N Bousquet et Sociorum
63 Happe H 1907 Peobachtungen an Eihauten junger menschlicher Eier Anat Hefte 32 171 213
64 Hartmann H 1929 Zur Feineren Histologie der Placentargefasse (nach gemeinsamen Untersuchungen mit H Runge) Arch f Gynak 137 730 75
65 Harvey W 1653 Anatomical Exercitations concerning the Generation of Living Creatures J Young London
66 Heinz R 1888 Untersuchungen uber den Bau und die Entwicklung der menschlichen Placenta Arch f Gynak 33 413 42
67 Hennig C 1872 *Studien uber den Bau der menschlichen Placenta* und uber ihre Erkrankungen Cited by Waldeyer W Arch f Mikr Anat 35 1 51
68 Herff O 1896 Beitrage zur Lehre von der Placenta und von den Mutterlichen Eihullen Zeitschr f *Geb u Gyn* Vol 35

69 Hicks B 1871 Some remarks on the anatomy of the human placenta Journal of Anat & Physiol 6 405 ibid 1872 On the contractions of the uterus throughout pregnancy their physiological effects and their value in the diagnosis of pregnancy Tr Obst Soc London p 215
70 Hinselmann H 1921 Subepitheliole Verdichtungsherde in Bindegewebe der Blasommalenzetten nebst einigen allgemeinen Bemerkungen zur Blasenmolenforschung Zeitschr f Geb u Gyn 83 313 35
71 Hoffbauer J 1924 Cited by A Mayer (1929) Biologie der Placenta I Arch f Gynak 137 25-34
72 Hoffmann G 1862 Sicherer Nachweis der sogenannten Uterinmilch beim Menschen Zeitschr f Geb u Gyn VIII 258 86
73 Hunter J 1754 Observations on Certain Parts of the Animal Oeconomy Nicol and Johnson London p 60
74 Iizuka S 1916 Uber Verkammen von Maskelfasern in der menschlichen Plazenta Beitr Geburt u Gynakol Vol 19 (Ergang heft) 101-3
75 Jacquemier J M 1838 Recherches d anatomie et de physiologie sur le systeme vasculaire sanguin de l uterus humain pendant la gestation et plu spe cialement sur les vaisseaux utero placentaires Arch gen de med Ser III T III p 165
76 Jailer J W and Knowlton A I 1950 Simulated adrenocortical activity during pregnancy in Addisonian patient J Clin Investigation 29 1430 36
77 Jassinsky P 1867 Zur Lehre Uber die Structur de Placenta Virchow s Archiv 40 341
78 Jones G E S 1954 The management of endocrine disorders of menstruation and fertility Charles C Thomas Co Springfield Illinois ibid 1956 The Placental Hormones Williams Obstetrics Eleventh Edition Appleton Century pp 169 80
79 Jones G E S Delfs E and Stran H 1954 Chorionic gonadotrophin and pregnanediol values in normal pregnancy Bull Johns Hopkins Hosp 75 356
80 Jones G E S Gey G O and Gey M K 1943 Hormone production by placental cells maintained in continuous culture Bull Johns Hopkins Hosp 72 26 38
81 Kostchenko N 1885 Das menschliche Chorionepithel und dessen Rolle bei der Histogenese der Placenta Archive F Anatomie und Physiologie von His und Braune und E du Bois Reymond Anat Abth 451 80
82 Kearns P J 1934 Anatomy and histology of placental circulation Amer J Obst & Gynec 27 870-77
83 Keibel F 1889 Zur Entwicklungsgeschichte der menschlichen Placenta Anatomischer Anzeiger Nr IV 537 9
84 Kiwisch F 1851 Die Geburtskunst mit Einschluss der Lehre von den ubrigen Fortpflanzungevorgangen im Weiblichen Organismus Betrage zur Geburtskunde IA 6th F Fuke Wurzburg
85 Klebs E 1873 Handbuch der Pathologische Anatomie bei K Rotikansky Wien Braunmuller and Seidel pp 929 30
86 Kolliker A 1861 Grundres der Entwicklungs-geschichte des Menschen und der hohren Tiere Leipzig I Aufl 1879 Leipzig II Aufl Lehrbuch d r Entwicklungsgeschichte des Menschen und der hohren Tiere 1884 Leipzig III Aufl Grundriss der Entwicklungsgeschichte des Menschen und der hohren Tiere
87 Krantz Kermit E and Arey L B 1950 The presence of smooth muscle in the human placenta and its apparent role in the circulation of the intervillous space Fdn Prize Thesis So Atl Assn Obst & Gynec
88 Krause A 1853 Die Theorie und Praxis der Geburtshulfe Reimer Publisher Berlin 155
89 Kupfer K 1888 Decidua des Menschen an enden des ersten Monats Munchener medic Wochenschrift Nr 31 365
90 Langhans T 1877 Untersuchungen Uber die menschliche Placenta Archiv f Anatomie und Physiol p 188
91 Lauth A 1826 Repertoire general d anatomie et physiologie I 75
92 Lee R 1838 Observations on the veins of the uterine decidua London Medical Gazette New Series I 334
93 Leonardo da Vinci 1500 III Organi della Generazioni Quaderni d Anatomi Fogli della Royal Library di Windsor
94 Leopold F 1677 Studien uber die Uterusschleimhaut wahrend Menstruation Schwangerschaft und Wochenbett Arch f Gynak 11 & 12 169 211 110 45
95 Lobstein J 1802 Cited from Handbuch der Entwicklungslehre der Wirbeltiere 1906 Bd I u II Teil by Hertwig Fischer Verlag Jena p 355
96 Lyon R A Simpson M E and Evans H M 1953 Qualitative changes in urinary gonadotrophins in human pregnancy during the period of rapid increase in hormone titer Endocrinol 53 674
97 McGill C 1907 The histogenesis of smooth muscle in the alimentary canal and respiratory tract of the pig Internat Monatschr f Anat u Phys 24 204 37
98 Magnus F Cited by O Grosser 1927 Fruhentwicklung Eihautbildung und Placentation Bergmann Melnchen p 340
99 Majnarich J J and Dillon R N 1954 Chromatographic evidences for a new corticoid in placental extracts Arch Biochem & Biophysics 49 247
100 Mallory F B 1906 Cited from Animal Micrology by M F Guyer Univ of Chicago Press p 172
101 Meyer Ruegg H 1929 Der Intervillose Raum im ersten Monat Zentralblatt f Gynak 53 1292 6
102 Mikulicz Radecki R 1929 Experimentelle Studien uber die Stromungsverhaltnisse im intervillosen Raum und in den Gefassen der Zotten Ztschr f Geburt u Gyn 156 1284
103 Millard C 1835 Connection of the uterus with the placenta London Med Gazette 14 654
104 Milligan M 1946 Trichrome Stain for Formalin Fixed Tissue Am J of Clin Path 10 184 5
105 Minot C S 1887 Bemerkungen zu dem Schroder schen Uteruswerke Anat Anzeiger II 1 199 ibid 1889 Uterus and embryo J of Morph 2 341 462 ibid 1892 Human Embryology published by Wood N Y pp 364 78
106 Mibuchi K 1924 Morphologische Studien uber das Verhalten der Nerven in den Weiblichen Geschlectsorganen des Menschen mit besonderer Berucksichtigung der Veranderungen ihres Verhaltens wahrend der Graviditat und Menstruation und im zunehmender alter Anhang Die Nerven in der Nebelschnur und Plazenta Mitteilungen aus der Medizinischen Fakultat der Kaiserlichen Universitat zu Tokyo 31 385-495
107 Monro D 1746 Edinburgh Physical Essays I 456 Cited by J Reid 1841 On anatomical relations of

the blood vessels of the mother to those of the fetus in the human species Edinburgh Med and Surg J No 146
108 Monro A 1747 Inmus Edinburgh Medical Essays 2 102
109 Monro A Secundus 1746 Edinburgh Physical Essays 1 481
110 Naujoks H 1922 Heben anatomische Veränderungen der kindlichen Eihäute einen Einfluss auf die Zeit des Blasensprunges Zeitschr f Geb u Gyn 84 304 34
111 Nitabuch R 1887 Cited from E Bumm 1893 Über die Entwicklung des Mutterlichen Placenta Arch f Gynak 37 1 15
112 Noble D 1834 The connexion between the uterus and the placenta London Med Gazette 14 810
113 Noortwyk W 1743 Uteri Humani Gravidi Anatome et Historia Lugd Batav p 10
114 Okkels H and Engle E T 1938 Studies on finer structure of uterine blood vessels of macacus monkey Acta Path et Microbiol Scandinav 15 150
115 Owens R 1841 Cited from J Reid On anatomical relations of the blood vessels of the mother to those of the foetus in the human species Edinburgh Med and Surg J 146 1 12
116 Pankow O H 129 XXI Tagung der Deutschen Gesellschaft fur Gynakologie zu Leipzig Zentralbl f Gynak 53 1603-4
117 Pap A 1936 Cited from J Wienbeck Beitrage zur Histologie und Physiologie der Plazenta zotte Ztschr f Mikr Anat Forsch 39 135 50
118 Pearlman W H and Cerceo E 1952 The isolation of the progesterone from human placenta Proc Endocrinol Soc J Biol Ch m 198 79 83
119 Phillipp E 1930 Hypophysenvorderlappen und Placenta Zentralbl f Gynak 54 450 2754
120 Pratt J P 1927 Corpus luteum in its relation to menstruation and pregnancy Endocrinol 11 195 208
121 Radford W 1832 Cited from J Reid Edinburgh Med and Surg J 146 1 12
122 Ramsbotham F 1834 Lectures on theory and practice of midwifery London Med Gaz tte 14 1 900 esp p 613
123 Ramsey E 1954 Circulation in the maternal placenta of primates Am J Obst & Gynec 67 1 14
124 Ramsey E M 1949 The vascular pattern of the endometrium of the pregnant rhesus monkey Carnegie Institute of Washington Publication #583 Contributions to Embryology 33 113 50
125 Reid J 1841 On anatomical relations of the blood vessels of the mother to those of the *foetus in the* human species Edinburgh Med and Surg J 146 1 12
126 Reynolds S R M 1954 Hemodynamics Characteristics of fetal circulation Am J Obst & Gynec 68 69 90
127 Robin M Cited from Cazeaus F and S Tarnier 1886 Theory and Practice of Obstetrics 8th Am Ed Blakiston pp 194 210
128 Rodway H E 1956 A study of Hofbauer *cells in* the human placenta J Obst & Gynaec Brit Emp 63 111 15
129 Romiti G 1880 Sulla struttura della placenta umana e pia specialmente sull natura del rivestimento del villi placentale Atti della R Accad della Fisiocritici Ser III 3 18-21
130 Ruge K 1886 Die Eihullen des *in der Geburt* befindlichen Uterus Bemerkungen *uber den Ort* und die Art d r Ernahrung des Kindes in demselben In dem Werke K Schroders H Der Sewangere und Kreisende Uterus Beitrage zur Anatomie und Physiologie der Geburtskunde Published by Max Cohen and Son Berlin pp 116 51
131 Sanger and Herff 1922 Cited from Naujoks Heben Anatomische Veran der ungen der kindlichen Ei haute einem Einfluss auf die Zeit des Blasensprunges Zeitschr f Geburt u Gynec 84 304 34
132 Schmitt W 1921 and 1924 Cited from O Grosser 1927 Fruhentwicklung Eihautbildung und Placentation Published by Bergmann Munchen p 341 ibid 1929 Uber die Blutbewegung in Placenta and Nebelstrang Zentralbl f Gynak 33 1232 92
133 Schott L 1836 Cited from Mobuchi 1924 Mitteilungen aus der Medizinischen Fakultat der Kaiserlichen Universitat zu Tokyo 31 385-495
134 Schroeder R 1929 XXI Tagung der Deutchen Gesellschaft fur Gynakologie zu Leipzig Zentralbl f Gynak 53 1799
135 Seiler E W 1832 Die Gebarmutter und das Ei des Menschen in den ersten Schwangerschafts menaten Dresden p 33
136 Spanner R 1930 XXI Tagung der Deutschen Gesellschaft fur Gynakologie zu Leipzig Zentralbl f Gynak 53 1799 ibid 1935 Randsinus und Randzone der Placenta Zentralbl f Gynak 59 1442 3 ibid 1935 Mutterlicher und kindlicher Kreislauf der menschlichen Placenta und seine Strombahnen Zeitschr f Anat u Entwickl 105 163 242 ibid 1940 Betrachungen zum Placentarkreislauf des Menschen Zentralbl f Gynak 64 2002 11
137 Spee G 1889 Beobachtungen an einer menschlichen Keimscheibe mit offener Medullarrinne und Canalis neurentericus Arch f Anat u Physiol von His und Braune u E du Bois Reymond Anat Abth p 160
138 Spivack M 1943 On the presence or absence of *nerves in the umbilical blood vessel* of man and guinea pig Anat Record 85 85-109
139 Stieve H 1946 Uber den Abfluss des *Blutes aus* dem Intervillosen Raum der menschlichen Placenta Zentralbl f Gynak 63 1570 82
140 Stoeckel W 1928 Zwei Fragen uber den Intervillosen Raum Zentralbl f Gynak 52 2578 84
141 Tafani A 1886 Sulle condizioni utereplacentari *della vita fetale* Mem delle Reale Institute de *studi superiori* practi i o di perfezionamento in *Firenze Cited by* Waldeyer Arch f Mikr Anat 35 1 51
142 Turner W 1872 Obser*vations on the structure of* the human placenta J Anat & Physiol 7 120 33 ibid 1877 Some general observations on the pla*centa with special* reference on the theory of evolution J Anat & Physiol 11 3 53
143 Van der Kolk S 1851 Maarnemingen over het *Maskel von de Menschlijka Placenta* en over haren *Blood omloop* Verhandling r van het K Nederlandsche Instituut
144 Vater A B Muller 1825 Diss qua uterus gravidus physiologee et pathologie el pathologiee considera tur exposita simul ejursdem structura sinusosa ac orificium menses Praesente A Vatero 4 13
145 Velpeau A 1833 Embryologie ou ovologie humaine *Paris Bailliere* pp 65 9 and the Introduction
146 Venning E and Browne J S L 1937 Urinary excretion of sodium pregnanediol glucuronidate *in* the menstrual cycle (an excretion product of progesterone) Am J Physiol 119 417
147 Venning E 1946 Adrenal function in pregnancy *Endocrinol* 39 203 20

148 Venning E Singer B and Simpson G A 1954 Adrenocortical function in toxemia of pregnancy Am J Obst & Gynec 57 542 8
149 Venning E Carballeria A and Dyrenfurth I 1954 Excretion of sodium retaining substances (abstract) J Clin Endocrinol & Metab 14 784
150 Vesalius A 1542 De Corporis Humani Fabrica Librorum Epitome
151 Virchow R 1853 Ueber die Erweiterung kleiner Gefasse Archiv fur pathologische Anatomie 2 449 ibid 1853 Ueber die Bildung der Placenta Gesamulte Abhandlungen p 779
152 Wagner G A 1929 Der Intervillose Raum Arch f Gynak 137 699 708
153 Waldeyer W 1890 Placenta Bau Menschen und Affen Archiv f Mikr Anat 35 1 51
154 Waldstein E 1929 Fruhkastration in der Schwangerschaft Zur Genesedes Ovarial Hormons Zentralbl f Gynak 53 1305-13
155 Weber E H 1832 Hildebrandt s Handbuch des Anatomie des Menschen 4th ed G F Walters Stuttgart 4 490 504
156 Werth R 1884 Beitrage zur Anatomie Physiologie und Pathologie der menschlichen Schwangershaft I Ueber die sogenannte Uterinsmilch des Menschen Arch f Gynak 22 233
157 Wienbeck J 1936 Beitrage zur Histologie und Physiologie der Plazenta zotte Ztschr f Mikr Anat u Forsch 39 135 50
158 Winkler K 1872 Zur Kenntniss der menschlichen Placenta Arch f Gynak IV Heft 2 238
159 Wislocki G 1943 The histology and cytology of the human and monkey placenta with special reference to the trophoblast Am J Anat 73 4 335-449
160 Wislocki G B 1948 Functional activities of placental trophoblast *Obst & Gynec Survey 3 604 14*
161 Wislocki G B and Dempsey E W 1955 Electron microscopy of the human placenta The Anatomical Record 123 133 49
162 Wrisberg H 1800 Commentationem Medici pp *46 and 312*
163 Zondek B 1931 Hypophysenvorderlappen HVH und Placenta Vergleichende quantitative Unlersuchungen bei Mensch und Tier Zentralbl f Gynak 55 1 12
164 Zondek B and Aschheim S 1928 Darstellung des weiblichen sexualhormons aus dem Harn insbesondere dem Harn von Schwangeren Klin Wchnschr 7 485
165 Hertig A T 1935 Angiogenesis in the early human chorion and in the primary placenta of the *macaque monkey Carnegie Institut Wash Publ* Contrib to Embryol 25 37 81
166 Hertig A T and Rock J 1941 Two human ova of the pre villous stage having an ovulation age of about 11 and 12 days respectively Carnegie *Institut Wash Publ 525 Contrib to Embryol* 29 127 56
167 —— 1945 Two human ova of the pre villous stage having developmental age of about seven and nine days respectively Carnegie Institut Wash Publ 557 Contrib to Embryol 31 65-84
168 —— 1949 Two human ova of the pre villous stage having a developmental age of about eight and nine days respectively Carnegie Institut Wash Publ 583 Contrib to Embryol 33 169 86
169 —— 1951 The implantation and early development of the human ovum Amer Jour Obs & Gynec 61A 8 14
170 —— 1944 On the development of the early human ovum with special reference to the trophoblast of the pre villous stage A description of seven normal and five pathological human ova Amer Jour Obs & Gynec 47 149 84
171 —— 1949 A series of potentially abortive ova recovered from fertile women prior to the first missed menstrual period Amer Jour Obs & Gynec 58 968 93
172 —— 1942 Some aspects of early human development Amer Jour Obs & Gynec 44 973 83
173 Heuser C H 1938 Early development of the primitive mesoblast in embryos of the rhesus monkey *Carnegie Institut Wash Publ 501 Cooperation* in Research pp 383-8
174 Heuser C Rock J and Hertig A T 1945 Two human embryos showing early stages of the definitive yolk sac Carnegie Institut Wash Publ 557 *Contrib to Embryol* 31 85 99
175 Heuser C and Streeter G 1941 Development of the macaque embryo Carnegie Institut Wash. Publ 525 Contrib to Embryol 29 15 55
176 Rock J and Hertig A T 1948 The human conceptus during the first two weeks of gestation Amer Jour Obs & Gynec 55 6 17

VI

Disturbances of Pregnancy

BARBARA J CALL, M D

PREGNANCY follows the general precept that for everything considered normal there will be certain deviations which must be considered abnormal For all of the numerous physiological changes associated with normal pregnancy there will be deviations from these physiological functions which will lead to disturbances in pregnancy and even to abnormal pregnancy

The importance of the role of hormones in pregnancy is beyond question but within the limits of our present biological and chemical assay methods there is a *normal range of values* to be considered rather than a single standard value for any hormone or hormone metabolite The wide variations found in normal pregnancy make the interpretations of these assays as applied to the disturbances of pregnancy somewhat confusing since the range of values found in abnormal pregnancy frequently overlaps the range of values determined for normal pregnancy The ultimate purpose of hormone assays is not to confirm the clinical diagnosis of a disturbed pregnancy but to predict the onset and degree of severity of the condition and direct us toward the best choice of treatment Hormone levels as found in the laboratory must be integrated into the clinical picture of any individual case before they can become useful and it becomes necessary to follow a trend rather than a single assay in evaluating the course of abortions premature labor diabetes and toxemia which are the chief causes of disturbed pregnancy

The placenta seems to play the leading role in the hormonal changes in pregnancy and many studies have been directed toward the placental production of estrogens progesterone and chorionic gonadotropin However the complete secretory activity of the placenta has not been determined and this temporary endocrine organ undoubtedly produces other protein and steroid hormones which may provide the solution to our present gestational problems Recent reports postulate that the placenta may produce ACTH like and adrenal cortical like hormones [7, 116] though relatively little is known concerning the hormonal interrelationships between placental function and that of the maternal and fetal adrenals Davis and Plotz [4] conclude that the balance established between the hormonal function of the placenta and the maternal adrenal cortex favors the development and the maintenance of normal pregnancy and that disturbances of this balance may lead to severe impairment of the gestational period although compensatory mechanisms exist which may overcome some of these disturbances without serious hazard to mother or baby

The lack of precise etiology of many of the disturbances in pregnancy makes the clinical application of hormones mostly arbitrary and experimental Further confusion is added when reports on good results from endocrine treatment are still matched by reports on equally good results from the use of general health measures rest and sedation The ultimate survival or defeat for endocrine therapy will depend on carefully arranged clinical studies with unbiased sampling valid statistical methods and adequate control groups The disturbances of pregnancy represent a very rich battlefield on which it remains to be seen whether hormone therapy will emerge victorious

THREATENED AND REPEATED ABORTION

Abortion is the term applied to pregnancies which terminate prior to the 28th week of gestation The exact incidence of spontaneous abortion is not known, but has been reported as varying from 10 to 20 per cent the incidence of threatened abortion likewise from 3 9 per cent by Rucker [9] to 26 per cent by Javert and Finn [56] The term repeated abortion is now frequently used in place of the commonly used term 'habitual abortion because it more accurately describes what is meant and refers to the woman who has had two or more consecutive spontaneous abortions either with or without an earlier *term* pregnancy Many investigators use three or more consecutive spontaneous abortions as the criterion for habitual abortion

The causative factors in abortion are varied fre

quently there is a combination of possible etiologies rather than a single cause, and frequently no cause at all is evident. In cases aborting during the first few weeks of pregnancy about 90 per cent reveal fetal defects incompatible with further development;[4] blighted ova account for about 46-48 per cent of abortions. Various endocrine effects probably represent the second most common cause, and the remainder may be due to local factors in the pelvis, infections, systemic disease, metabolic disturbances, and even other factors as yet unknown by our present methods. In evaluating the present status of therapy in abortion, King[60] states that the confusion in the recent literature in regard to abortion is due to differences in the selection of cases treated, differences in definition of threatened and repeated abortion, conclusions from an inadequate number of cases without adequate controls, and failure to consider the element of chance. Various programs of hormonal therapy have been used, as well as vitamins, bed rest, psychiatric treatment, and no treatment, but not one of these methods has proved exclusively superior to any other method of treatment. In reviewing 2792 cases of threatened abortion, as reported by nine authors, King found that 67 per cent (standard error 0.28 per cent) carried to term regardless of the treatment or the absence of it, and that of 1820 cases of repeated abortion reported by 14 authors 61 per cent (standard error 1.14 per cent) carried to term regardless of the type or absence of treatments.[60]

METHODS OF HORMONE EVALUATION

Hormonal levels in pregnancy reflect to a certain extent the functional state of the trophoblast. Any disturbance or cessation of trophoblast function leads to diminished hormone secretion and a decrease in hormone levels, as evidenced by quantitative bioassays and chemical methods. In serial determinations of hormone levels, an early drop or a consistently decreasing hormone level may occur when clinical symptoms are few or absent, and in these cases eventual miscarriage is apparently due to hormonal disturbances.[14]

Estrogen

Assays of urinary and serum estrogen have not been widely used although the excretion decreases rapidly in cases of inevitable abortion or fetal death.[103, 104, 105] Jayle[7] believes that the determination of the amount of estrogen present in cases of habitual abortion is important, and that the demonstrated insufficiency of estrogen output is much more serious and of greater prognostic value for the pregnancy than the determination of pregnanediol. Further studies on estrogen excretion are indicated.

Progesterone

Progesterone is excreted in the urine as pregnanediol, and the quantitative evaluation of urinary pregnanediol by serial studies has been advocated as a guide to therapy and prognosis in threatened abortion.[39, 45, 123] The clinical significance of pregnanediol excretion in disturbed pregnancy has been studied extensively, but is still a highly controversial subject. As a result of their laboratory investigations and clinical studies, the Smiths and their co-workers reported[10, 11] that diethylstilbestrol administered to pregnant women resulted in an increased excretion of pregnanediol as measured by the Venning method[1, 3] when the placenta was capable of being stimulated to increased secretory activity, and concluded that estrogen was essential for the normal metabolism and physiological utilization of progesterone. Their work has been confirmed by Jayle[57] and others. However, Davis and Fugo,[2] measuring pregnanediol in the free state rather than the sodium pregnanediol glucuronide complex of the Venning method, failed to confirm the Smiths' finding that stilbestrol increased pregnanediol excretion. Sommerville and his co-workers,[1, 4] employing a colorimetric procedure for the measurement of pregnanediol in acid boiled urine, obtained results diametrically opposite to the Smiths. Pearlman et al.,[81] experimenting with the administration of deuterium labeled estrone, also failed to support the Smiths, particularly with respect to the influence of progesterone on the course of estrone metabolism in human pregnancy. These observations have led to doubt concerning the value of stilbestrol in pregnancy, since the results seem to vary with the particular method used for measuring urinary pregnanediol. Using a pregnanediol test based on a modification of the Astwood-Talbot technique,[4] Guterman found the average minimal level of excretion in normal pregnancy to be 5 mgm. per 24 hours. In following 148 patients with threatened abortion in the first trimester,[41] he found 86 per cent retention and 14 per cent abortion when the pregnanediol excretion trend was normal (5 mgm. or more), and only 10 per cent retention with 64 per cent abortion when pregnanediol excretion trend was less than normal. Koff and Tulsky,[8] in following 335 cases, found 97 per cent of their patients aborted when there was a downward trend of pregnanediol excretion, whereas only 21 per cent aborted when the excretion was greater than normal. These findings in general have been confirmed by others[7] and demonstrate the value of pregnanediol levels in determining the need for progesterone in therapy and the prognostic outlook in threatened abortion.

Chorionic Gonadotropin (HCG)

Quantitative methods for measuring urinary HCG are more valuable than qualitative methods since the latter test may remain positive despite deficient trophoblast activity if the decreased HCG level is still above the low threshold usually tested Measurement of HCG is the only specific test of the activity of the trophoblast From a series of 500 cases Zondek *et al*[133] concluded that the rough titration of urinary HCG was of diagnostic value in threatened and missed abortion Behrman and Nieman[10] have recently reported on a method for the quantitative and qualitative analysis of HCG in the serum and made the interesting observation that *HCG disappears* from the serum 1-4 days before the urinary rapid rat test for HCG becomes negative this has practical significance in the treatment of threatened and inevitable abortion

Cervical Mucus Arborization

Zondek investigating the arborization phenomenon of cervical mucus described by Papanicolaou in dry smears from the estrogen phase of the menstrual cycle[77] found that the formation of fern or palm like (PL) pattern occurred only when the cervical mucus was under the influence of estrogen unopposed by progesterone activity He further observed that the inhibiting effect of progesterone on fern formation could be overcome in the premenstrual phase by the administration of estrogens This could not be accomplished during pregnancy suggesting that a special mechanism exists in pregnancy which inhibits the excretion of mucus containing the electrolytes necessary for the arborization pattern[22] On the basis of cervical mucus smears Zondek *et al*[15] reported that a slightly positive PL reaction during pregnancy may indicate functional insufficiency of the placenta which can be irreversible leading to abortion or reversible with continuance of the pregnancy as the full function of the placenta is restored While the mechanism of arborization during pregnancy is not clearly understood the cervical mucus smear is easily obtainable and simple to interpret, and will undoubtedly prove very helpful in the management of the abortion problem

Cervical Cytology

Cervical smears stained according to the Papanicolaou technique provide a practical and reliable measure of the relative adequacy or deficiency of progesterone effect during pregnancy Progesterone effect is adequate when nearly all the cells take the basophilic stain and there is practically no cornification present Randall and his co-workers[87, 88] using this technique since 1949 have obtained smears from over 3000 women during the early weeks of pregnancy By showing progesterone deficiency these smears have repeatedly predicted impending abortion before any clinical signs or symptoms appeared, and have proved reliable in the early detection of missed abortion When cytology in early pregnancy indicated adequate progesterone in 2541 pregnancies 92 6 per cent terminated with a live child and about 5 per cent aborted with no treatment In 258 gravida 3+ (three or more pregnancies) with a good obstetric history and poor smear 92 1 per cent had a live child In the 311 patients whose smear showed a relative deficiency of progesterone 48 7 per cent terminated in a live child and 24 1 per cent in abortion Of 41 gravida 3+ patients with a poor obstetrical history and good smear 85 per cent of these pregnancies terminated in a live child in 33 gravida 3+ patients with a good obstetrical history and a poor smear 72 7 per cent terminated in a live child Of 10 gravida 3+ patients with a poor obstetrical history and a poor smear no live child was produced in 40 pregnancies Randall s group also managed 49 habitual aborters 9 were treated with stilbestrol (Smith s regime) and 10 became pregnant on Hughes s preconceptional treatment[5] 30 were given no hormones after conception, and 19 delivered live babies *There was no significant difference between the two groups 63 2 per cent of babies surviving* in the treated group and 63 3 per cent in the not adequately treated group Furthermore 62 per cent of the abortions in Randall s series occurred in spite of a good smear and this casts doubt on the prognostic value of the procedure Only 38 per cent of those who aborted had poor smears suggesting that sex steroid deficiency is only one of the factors to be considered in abortion Finally the use of stilbestrol according to the Smith regime did not reduce the incidence of abortion when smears suggested no hormonal deficiency

PREGNANCY PROGNOSIS IN REPEATED ABORTION

For years the figures arrived at by Malphas[4] and Eastman[9] have been used as the standard for the prognosis of repeated abortion but the accuracy of their estimates is questionable According to Malphas the spontaneous cure rate after three successive abortions is only 27 per cent Eastman s calculated rate is 16 4 per cent These high rates according to Speert have resulted from the uncritical acceptance of poorly founded estimates of the frequency of recurrent causes in his study of a series of 121 women[15] with a history of three or more consecutive abortions 81 per cent carried their pregnancies to viability and of 66 women with three consecu

tive abortions 89 per cent carried their pregnancy to viability He further found that only 65 per cent of the hormonal treated patients went to viability as compared to 85 per cent of the untreated patients and felt that the good results could not be ascribed to hormone therapy Without the benefit of any hormonal therapy Bevis reported that in his group of 32 women with three or more previous abortions 29 patients (91%) reached the 28th week of pregnancy and 26 (81%) of the babies were born alive and well His treatment was aimed at gaining the confidence of his patients and advising them to take extra rest at the time of their menses[14] From these and other studies it would appear that the outlook in habitual abortion is much more optimistic than the figures of Malphas and Eastman indicated

TREATMENT OF THREATENED ABORTION

No specific therapy has been developed to prevent abortion and the present status of therapy in threatened abortion is very confusing Colvin *et al*[20] attempted to show the futility of specific therapy in abortion by reporting the salvage possibility of 1570 cases of threatened abortion (Fig 1) 69 9 per cent continued to term 20 3 per cent aborted blighted ova 3 8 per cent aborted due to pathology of the *fetus placenta* or membranes and 2 1 per cent ended in premature labor These represented 96 1 *per cent of cases in which treatment with the various* hormones would not have influenced the results and left only 3 9 per cent or 62 cases which might have benefited from specific treatment They have advocated bed rest and sedation with reasonable *activity* as to meals and bathroom privileges until such time as the patient improves or aborts also if the life or death of the embryo could be established treatment could be given in a more intelligent manner

Diddle and his co workers in evaluating bed rest versus conservative ambulation in 1452 cases of threatened abortion[26] found no appreciable difference in the incidence of spontaneous abortion The chief value of bed rest appeared to be in the control of bleeding rather than the ultimate fate of the pregnancy and it was apparent that prolonged bed rest in the treatment of threatened or habitual abortion could be easily abused

In 1948 Smith reported on the use of small doses of diethylstilbestrol in threatened abortion[109] and had a 78 per cent cure rate for 219 cases Two other

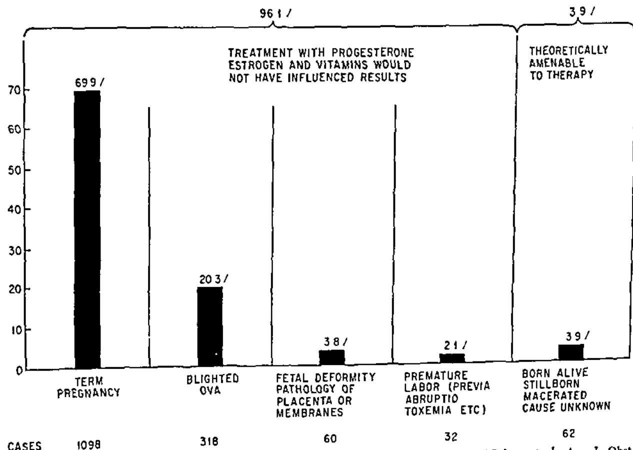


FIG 1 SALVAGE POSSIBILITIES IN 1570 CONSECUTIVE CASES OF THREATENED ABORTION (Colvin *et al* Am J Obst & Gynec 59 1208 1950)

studies on the use of stilbestrol [2, 91] employing good control groups found that the stilbestrol treated patients did no better than the control patients numerous other studies have failed to confirm the usefulness of stilbestrol in threatened abortion and even the Smiths now stress its usefulness only when used prophylactically and not after the onset of symptoms

Progesterone in large and small doses gave reported cure rates from 58 to 65 per cent [43, 63, 91] which are no better than those reported for no treatment The use of progesterone is recommended only in those cases for which a progesterone deficiency can be demonstrated and even then it will not salvage all of those cases

It appears that in threatened abortion survival of the pregnancy is good whether or not treatment is given Observation conservative ambulation and the use of specific therapy when deficiencies are evident seems to be the most logical approach to the treatment of threatened abortion

TREATMENT OF HABITUAL OR REPEATED ABORTION

Various hormones and combinations of hormones have been used in the treatment of habitual abortion For the success claimed with treatment there have been several cases who have subsequently carried a pregnancy when no treatment was employed It would almost seem advisable to consider each pregnancy on its own merits rather than to blame recurrent factors for subsequent abortions in view of the number who eventually achieve a viable pregnancy without treatment

Thyroid has long been used empirically alone or as a supplement in the treatment of habitual abortion *Thyroid activity gauged by the protein bound* iodine determination (PBI) of the serum was found to be more accurate for evaluating thyroid function than the basal metabolic rate (BMR) 4 0 8 0 gm / 100 cc of serum was judged normal 4 0 was considered low normal and 7 5 8 0 high normal Table 1 shows the effect of thyroid medication on habitual aborters as studied by Comminos [21] In this limited series of cases there is no evidence that exogenous thyroid medication is of any benefit to habitual aborters whose PBI *is truly normal* In general it would seem that thyroid should be used only when there is clinical or laboratory evidence of thyroid deficiency

Smith and Smith on the basis of their experimental evidence that stilbestrol is capable of stimulating more normal secretion and *metabolism* of progesterone have stressed the prophylactic use of diethylstilbestrol in habitual abortion In their series of 81 habitual aborters (3 or more *successive spon*taneous abortions at 6 20 weeks) 60 patients or 74 per cent have had viable infants [107] The dosage of diethylstilbestrol began with 2 5 mgm daily when started at 5 weeks 5 mgm at 7 weeks 10 mgm at 9 weeks 15 mgm at 11 weeks 20 mgm at 13 weeks 25 mgm at 15 weeks and a 5 mgm increase weekly thereafter until four weeks from term when therapy was discontinued *The effectiveness of their treatment* depends on a trophoblast capable of responding since stilbestrol will not maintain a defective trophoblast Dieckmann's group [5] and Davis and Fugo [23] found that stilbestrol did not reduce the incidence of abortion and many others have failed to reproduce the good results of *the Smiths with the use of stilbestrol*

Table 1 EFFECT OF THYROID MEDICATION ON HABITUAL ABORTERS

(24 Thyroid treated 28 Nonthyroid treated—Comminos Obst & Gynec 7 260 1956)

	PBI level				
	Thyroid treated			No thyroid treated	
	Normal	Low normal	Low	Normal	Low normal
No patients	10	8	6	22	6
No pregnancies	4	0	3	13	0
FTD	3	0	1	10	0
Per cent	75 0		33 3	76 9	

Guterman [4] was not able to demonstrate a persistent decrease in progesterone secretion prior to the symptoms of threatened abortion in patients with repeated abortions nor was there *any improve*ment in pregnancy survival among the group who received 50 100 mgm progesterone I M daily as opposed to his control group Success rates of 72 and 74 *per cent* have been reported with implantations of six 25 mgm progesterone pellets [1, 117] in habitual abortion but the success rates *were almost equally* as good in their nontreated habitual aborters Again these findings point out that progesterone deficiency *is only* one of the many factors involved in abortion so that progesterone replacement *does not guarantee success* even in those cases which lack progesterone Combinations of estrogen and progesterone with and without thyroid or vitamin E *have been tried without any* consistent improvement over untreated *groups*

Lower levels of chorionic gonado*tropin were found in abortion in some instances* accompanied by normal *or falling* pregnanediol levels also cases were noted with low levels for both chorionic gonado

Table 2 DATA ON ENDOCRINE TREATMENT IN SIX CASES

(Wilson Am J Obst & Gy ec. 69 614 1955)

Pregna cy No	Date	Estroge mg per day	Progesterone mg per day	Thyroid gra ns per day	Cho o c Gonadotrop I.U	O tcome
Case 1						
1	2-45	0	0	0	0	Abort on 2½ mo ths
2	8-45	1(S)	10-20(AP)	0	0	Abort on 5½ months 20 cm fetus
3	5-46	0	0	0	0	Abort on 2 ½ months
4	6-47	3(S)	15(AP)	½	0	Abortion 2½ months
5	6-48	5-100(S)	150(AP)	0	0	Abort on 3 months
6	2-49	0	60(AP)	½	0	Abort on 3 months
7	2-51	2 5-3 75(CE)	0	½	4000 q 2-3 days later o ce a week	Normal i fant 38 weeks; 3 160 g ams
Case 2						
1	1946	0	0	0	0	2 190 gram nfa t 33 weeks; l ved
2	1947	0	0	0	0	Abortion 4 ½ month
3	1948	2-50(S)	20-30(AP)	0	0	Abort on 5 month
4	1950	15-125(S)	0	0	0	Abort on 5 month
5	1952	2 5-15(CE)	30-100(AP) 100(BP)	0	4000-5000 q other day	3 560 gram fa t; normal
Case 3						
1	1942	0	0	0	0	No mal i fa t 40 w eks 3 150 g am
2	3-49	0	0	0	0	Abort o 2½ mo ths
3	9-49	25(S)	30(AP)	0	0	In omplete abort o 2 ½ mos. cu ttage
4	5-51	0	0	0	0	Abortion 2 months
5	1952	2 5-7 5(CE)	25(BP)	0	4000 q other day	Normal nfa t 42 weeks; 3 670 g am
Case 4						
1	1944	0	0	0	0	Abort o 2½ mo th
2	1945	0	0	0	0	Abort o 5 mo ths
3	1948	0	0	0	0	Abort on 2 mo ths
4	1949	3-5(S)	20(AP)	1	0	Abort on 2½ months
5	1953	5-15(CE)	25-50(BP)	0	4000 q other day	Normal i fa t 39 w k ; 2 530 g am
Case 5						
1	1-51	0	0	0	0	Abort o 2 months
2	6-51	0	0	0	0	Abort on 2 months
3	12-51	5(S)	20(P)	½	0	Abortio 2 months
4	1953	3 25-20(CE)	50-75(AP)	½	4000 q other day	No m l fa t 41 weeks; 3,760 g am
Case 6						
1	1949	0	0	0	0	No mal i f nt 41 w eks 3 500 gram
2	1950	5(S)	0	0	0	Abort on 2 ½ mo ths
3	1951	15-50(S)	0	2	0	Abort o 2 mo th
4	1953	0	0	2½	0	Abortion 2 ½ mo th
5	1954	5-7 5(CE)	30(AP)	2½	4000 q oth r day	Normal i fa t 40 week ; 3 770 grams

* Abbre t o AP anhyd ohyd o yprogestero e BP buccal p og t ro e; CE, co jugat d e t ogen (eq) P proge t one tr mus ularly; S st lbestrol

tropin and pregnanediol[58] Chorionic gonadotropin (HCG) has not been used extensively probably because of local and systemic reactions incurred in the use of the available equine gonadotropin preparations It was felt that the normal level of HCG was so high in normal pregnancy that sufficient amounts of even human CG could not be given to reach these normal levels However with the availability of human CG (Antuitrin S—Parke Davis) and the idea that such therapy is largely substitution and not *replacement it would seem that repeated small doses* of human CG could be given where indicated by low HCG levels and would salvage a few more infants in cases of repeated abortions[15 17 58 97]

Estrogen progesterone and chorionic gonadotropin have been administered to six habitual aborters by Wilson with six normal infants representing 100 per cent success[81] These six patients represented 25 previous pregnancies 22 of which ended in abortion The data on the endocrine treatment in these cases are shown in Table 2 Wilson based his treatment on the concept that endocrine substances can assist the human female in her efforts to maintain a normal environment for the normal fertilized ovum and that the extreme fluctuations in endocrine production can be minimized While it is not certain that his results are due to the endocrine therapy the frequent endocrine abnormalities found in thoroughly studied patients do provide a rational basis for the use of hormone therapy

It has been suggested that much of the success claimed by all methods of endocrine therapy might be due to the psychological effect on the patient that she was receiving help Javert and Berle[11] pointed out the stress factors associated with habitual abortion and Javert[5] reported an average salvage rate of 91 per cent with his program of current therapy and psychotherapy exclusive of hormones in 100 cases of habitual abortion The psychiatric aspects of this problem are under further investigation[6] and the value of psychotherapy in habitual abortion remains to be shown

In summary no method of treatment of either threatened or repeated abortion has proved exclusively superior to any other method of treatment or lack of treatment Since treatment will change the outcome in only a small percentage of cases hormonal therapy should be reserved for those cases showing definite evidence of hormone deficiency *Large carefully controlled series of cases are needed for the final evaluation of therapeutic agents in abor*tion which means a pooling of results obtained from cases handled by the same scientific methods for all investigators Progesterone and chorionic gonadotropin seem to be the most logically used *hormones in threatened and repeated abortion due to hormonal* deficiency while estrogen appears *most valid* in preconceptual stimulation of a poorly developing endometrium Thyroid should be used only when there is evidence of hypothyroidism Finally there is little doubt that threatened and repeated abortion creates a stress reaction and the psychiatric investigations in this field can be expected to shed some light on this problem of abortion

TOXEMIA OF PREGNANCY

Toxemia is the generalized term applied to a group of diseases occurring during late pregnancy or the early puerperium It is characterized by one or more of the following signs edema albuminuria hypertension convulsions or coma and there may also be gastrointestinal cerebral visual and renal symptoms More specifically the terms pre-eclampsia and eclampsia are used to describe the condition

In spite of numerous investigations into the endocrine and metabolic changes associated with toxemia the etiology is unknown and most of these changes must be presumed to be secondary to the underlying causes Since toxemia never occurs in the absence of placental tissue it seems logical to blame placental dysfunction for the endocrine imbalances found

The placental hormone patterns in toxemia have been extensively studied and reviewed by Smith and Smith[102 105 6] They have done quantitative studies on some or all of the female hormones in normal pregnancy pre eclampsia and eclampsia (Fig 2) In a very high percentage of their cases there were high levels of chorionic gonadotropin and decreased excretion of estrogens and pregnanediol associated with degeneration of the syncytial cells of the placenta In all these cases these changes were found to precede any clinical signs of *pre eclampsia—eclampsia*

In 1949 the Smiths[11] reported on the prophylactic use of diethylstilbestrol on the progress and outcome of pregnancy on 387 primigravidas since *this group was generally found to have a high incidence* of late pregnancy complications their control group of 555 patients received no special treatment They concluded that the incidence of toxemia and the total fetal mortality were significantly lower in the treated mothers Incidence of toxemia was 2 3 per cent in the stilbestrol treated *mothers and* 6 8 per cent in the control *group the neonatal mortality* was 1 per cent in the treated and 3 8 per cent in the controls If spontaneous *premature delivery oc*curred in the stilbestrol treated mothers the premature infants were unusually large and *mature for* their gestational age Their failure to administer a placebo to their control group has led to the criticism of their report on the basis of an inadequate control group since other investigators were not able to

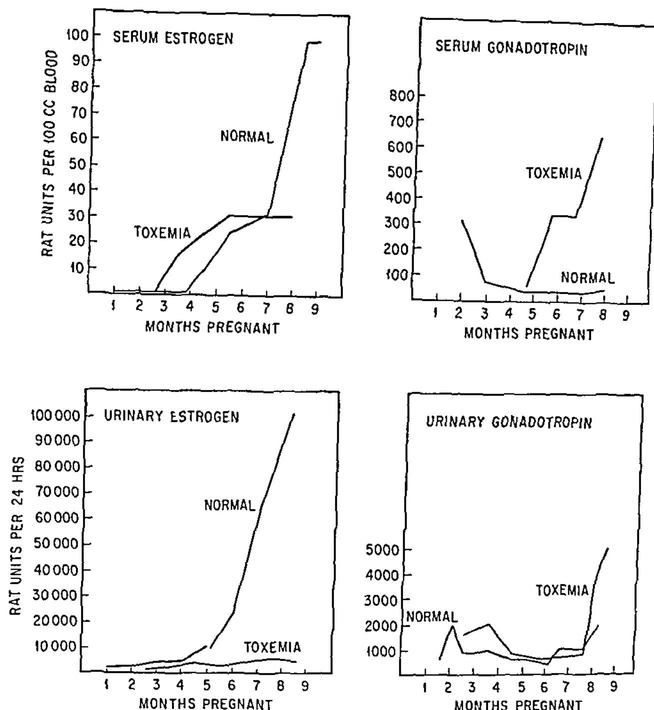


FIG 2 GRAPHS ILLUSTRATING THE ESTROGEN AND GONADOTROPIN LEVELS FOR NORMAL AND TOXEMIC PATIENTS (From Dieckmann The Toxemias of Pregnancy 2nd ed St Louis 1952 The C V Mosby Company modified from Smith and Smith Am J Obst. & Gynec 33 365 1937)

duplicate their results [28, 32] Dieckmann and his group carried on a strictly controlled clinical trial on the administration of diethylstilbestrol to 840 patients while an identical placebo tablet was given to 806 patients their group included multiparas as well as primiparas In their study stilbestrol did not reduce the incidence of pregnancy toxemias nor perinatal mortality premature babies of stilbestrol treated mothers were no longer or more mature for their gestational ages than the prematures of placebo treated mothers Ferguson also using multiparas as well as primiparas had 184 stilbestrol patients and 198 placebo patients who completed the dosage schedule or delivered while on it No cases of eclampsia developed in either group The incidence of pre eclampsia was 10 3 per cent in the stilbestrol group and 10 1 per cent in the placebo group he concluded that stilbestrol had no effect on pre-eclampsia

prematurity fetal weight and survival or size of the placenta The Smiths[107] do not feel that these results are comparable to their own because heterogeneous groups were studied which were not large enough to show significant differences furthermore they limited toxemia to pre eclampsia–eclampsia whereas Dieckmann and Ferguson also included essential hypertension and nephritis as toxemias the Smiths do not claim that stilbestrol is a panacea in the treatment of pregnancy disturbances but do feel that their results continue to indicate that stilbestrol administered prophylactically is reducing fetal wastage

Regarding essential hypertension and kidney disease one must be on the lookout for a superimposed toxemia As with toxemia the use of stilbestrol in these cases has little if any prophylactic value

In 1940 Selye[99] suggested that toxemia of late pregnancy could be regarded as a disease of adaptation caused by continued hyperfunction of the maternal adrenal cortex and many others have adopted this hypothesis However Mastboom[69] presupposed the possibility of an extra adrenal production of hormones with cortico-steroid structure especially of the desoxycorticosterone (DOCA) group in toxemia and to a lesser extent in normal pregnancy Since toxemia depended on a placenta but not necessarily a fetus the placental production of hormones with an adrenocorticoid structure seemed logical The possibility that the fetal adrenals produced an increased elaboration of these hormones in toxemia has not been substantiated[61] Characteristic of toxemia and DOCA intoxication were hypertension edema and albuminuria both syndromes increased in severity with a sodium rich diet It was thought an increased elaboration of sodium retaining corticosteroids (mineralocorticoids) might be an etiological factor in toxemia but administration of large doses of DOCA and salt failed to produce toxemia in normally pregnant women and the administration of DOCA plus salt did not cause any significant increase in weight B P Na or K balances in toxemic patients[5 27] Venning observed both a qualitative and a quantitative change in the excretion of urinary corticoids in toxemia her group[124] found a decrease in the glucocorticoids which affect carbohydrate metabolism and an increase in the level of the sodium retaining factor (SRF) in all cases of toxemia as shown in Figure 3

Many others reported similar findings[19 37 1 0] though again there was an overlapping of levels between the normally pregnant and the toxemic patients and a higher SRF was found many times in mild pre eclampsia over severe pre-eclampsia The elevated SRF level fluctuated within wide limits throughout the course of the toxemic state and usu

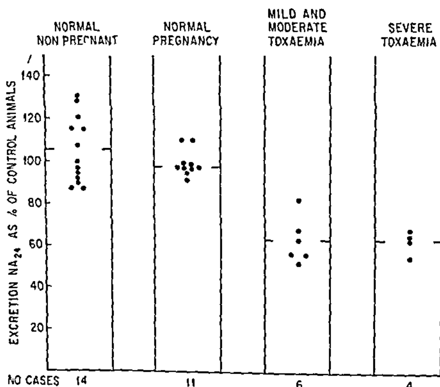


FIG 3 SODIUM RETAINING FACTOR (Venning *et al* Am J Obst & Gynec 67 542 1954)

ally disappeared within 48 hours following delivery Furthermore it has been shown that the SRF is generally increased but may be decreased in pre eclampsia also the degree of sodium retention does not bear a direct relationship to the severity of the clinical syndrome [1, 9] The *source and mechanism of* production of the SRF under both normal and toxemic conditions are unknown although a substance has been identified in adrenocortical extracts which shows strong sodium retaining potency and is clearly not DOCA This sodium controlling hormone of the adrenal cortex has subsequently been isolated and called aldosterone aldosterone has also been identified in the urine of patients with pre eclampsia and eclampsia *as well as in other edematous conditions* such as heart failure nephrosis and cirrhosis of the liver it has the most vigorous sodium retaining action of any of the steroids and although sodium retention is its most conspicuous effect it also results in the elimination of potassium While aldosterone *forms a part of the normal mechanism for electrolyte* control studies suggest that it is a sequel and not the cause of edema in the eclamptogenic toxemias its role as an eliminator of potassium in toxemia demands further investigation

Many studies concerning the role of the antidiuretic substance from the posterior lobe of the pituitary gland in the toxemias of pregnancy have been carried out since this hormone causes water retention and usually sodium and chloride excretion Hughes and his co workers found no increase in antidiuretic activity of serum during normal and toxemic pregnancy [51] Some studies reported an increased plasma level of the antidiuretic substance in toxemic patients others failed to demonstrate an antidiuretic substance in this condition and still others failed to show a correlation between antidiuretic activity and toxemia of late pregnancy [3, 44, 74]

The elevated levels of 17 ketosteroids during normal *pregnancy are undoubtedly due to a general* increase in the activity of the maternal adrenal cortex but their further increase in pre eclampsia and eclampsia rather than being etiological in toxemia probably results from an increased production of placental ACTH or some other adrenocortical stimulating substance which appears in toxemic pregnancies [129]

Moore and his group [73] were the first to report on the use of adrenal cortical hormones in the treatment of toxemia and claimed considerable clinical improvement in their cases as did Tew and McAlpine [101] However improvement was probably due to the euphoria caused by cortisone since B P levels and albuminuria showed no significant alteration during *treatment Many other studies* have failed to confirm these preliminary favorable results [2, 7, 8] Margulis *et al* concluded that ACTH and/or cortisone demonstrated no favorable *influence upon toxemia* that they did not produce symptoms of toxemia and finally that pregnant patients showed an increased tolerance to ACTH and cortisone Any improvement *in toxemic patients receiving ACTH* or cortisone was hardly more than could be expected from the usual hospital program of bed rest sedation and the limitation of sodium chloride and fluids

In conclusion we must look beyond the known hormones of the adrenal gland and the placenta for the etiology of toxemia and continue with conservative symptomatic measures rather than hormonal therapy in the treatment of pre eclampsia–eclampsia

HYPEREMESIS GRAVIDARUM

Hyperemesis gravidarum or pernicious vomiting of pregnancy is frequently considered a toxemia of early pregnancy Undoubtedly the early months of *gestation are filled with emotional as well as physio*logical adjustments and in most cases of hyperemesis gravidarum there is a psychic component [46] usually centered around disorders of sexual adjustment and fears related to pregnancy

In 1932 Kemp proposed his theory of adrenocortical insufficiency as the etiological factor and Carreras [18] viewing the coincidence of symptoms felt that hyperemesis gravidarum is a manifestation of the syndrome of general adaptation formulated by Selye During the early weeks of pregnancy the increased secretion of pituitary gonadotropins would lead to a temporary deficit of adrenocorticotropic hormone with the production of a transitory adrenal insufficiency the hormones produced by the placenta would neutralize the excessive pituitary gonadotropin and establish hormonal equilibrium between the fetus and mother ACTH production becoming normal or even high and the adrenal cortex *secreting adequate amounts of other* hormones If this neutralization process is not completed the pituitary then produces large amounts of ACTH and the adrenal cortex continues to throw off steroid hormones *whose properties could account for the* signs and symptoms of toxemia

Hyperemesis has also been considered as an allergic manifestation since adrenocortical insufficiency causes *hypersensitivity to histamine* [124] *gonadotropic* hormone is believed to be the responsible allergen Chorionic gonadotropin levels have been reported higher in this condition than in normal pregnancy and are correlated *with the severity of the clinical* signs of the condition [96]

Using the rationale of adrenocortical insufficiency ACTH and cortisone have been used in the treatment of hyperemesis gravidarum and *good results*

reported[18 122 126] ACTH given I V (15 mgm in 500 cc 5% D/W daily) relieved 15 patients completely in 2 4 days without aftereffects and with normal babies at term Cortisone in dosage of 25 75 mgm daily was administered to 29 patients who improved with some recurrences duration of treatment ranged from 5 days to 3 weeks three babies in this series had congenital defects which did not seem related to cortisone administration

While cortisone and ACTH have relieved some patients with pernicious vomiting results have been equally good with sustained hydration rest and sedation including placebos Anti nausea drugs have proved equally good in improving these patients It *would seem that these conservative methods provide* a safety factor which obviates the need for using such potent drugs as ACTH and cortisone in hyper emesis gravidarum

PREGNANCY AND DIABETES

The survival of the fetus has become the main objective in the problem of diabetes and pregnancy In spite of insulin and good medical obstetrical pediatric care the fetal loss still averaged about 30 per cent[59 64 1 1] Maternal factors influencing fetal loss *were spontaneous abortions acidosis and tox emia* which included not only pre eclampsia and eclampsia but also edema albuminuria and hyper *tension* which may be present even before preg nancy Fetal factors contributing to fetal loss were increased size of the baby prematurity neonatal hypoglycemia and congenital malformations

DeCosta has illustrated the present status of treatment in pregnant diabetics according to Figure 4

The army is made up of the White the Gray and

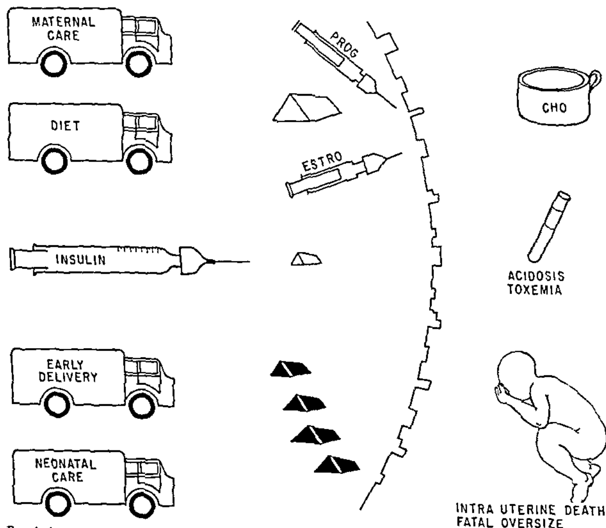


Fig 4 Analogical representation of army poised for attack against enemy (diabetes and complications) (DeCosta Obst & Gynec 5 401 1955)

Table 3 CLASSIFICATION OF DIABETIC PATIENTS AS TO RISK OF PREGNANCY

(White *et al* Am J Obst & Gynec 71 57 1956)

Class A—Diabetes shown by glucose tolerance test
Class B—Diabetes with onset after age 20 duration 0–9 years and no vascular disease
Class C—Diabetes with onset between ages of 10 and 19 duration 10–19 years and no vascular disease
Class D—Diabetes with onset before age 10 duration 20+ years and vascular disease evidenced by calcification of leg arteries and retinopathy
Class E—Diabetes with calcified pelvic *arteries*
Class F—Diabetes with nephropathy

Table 4 DAILY INTRAMUSCULAR DOSEAGE OF STILBESTROL AND PROLUTON (IN MG)

(White *et al* Am J Obst & Gynec 71 57 1956)

Week of pregnancy	Class				
	B	C	D	E	F
0–16	25	25	25	25	25
17–19	25	25	50	50	50
20–23	50	50	100	100	100
24–29	100	100	125	125	125
30–33	100	100	150	150	150
34 to end	125	125	200	200–250	200–250

the Black divisions who have a common objective but no controlling policy All use good maternal and fetal care proper diet insulin and early delivery to reach their objective but differ in organization philosophy and choice of auxiliary weapons [5] The White division [75 1 1 8] considers the use of estrogen and progesterone very important the Black division [83 3 59 8] does not use hormones and the small Gray division also uses hormones but in other than the recommended dosage [72 84 107]

For years the White division headed by Priscilla White at the Joslin Clinic has advocated and emphasized the need of *hormone therapy* and has come to five general conclusions First that an imbalance of female sex hormones occurs in abnormal pregnancies in diabetic patients low levels for serum estrogen low levels of pregnanediol excretion and high levels for chorionic gonadotropin have been the common hormonal pattern Secondly that this imbalance predicts or precedes the complications of pregnancy Thirdly that the imbalance can be corrected by the administration of female sex hormones which should be administered by the intramuscular route Fourthly that such correction leads to improved fetal survival provided other parts of management of pregnancy and diabetes are observed And finally that the imbalance occurs more often and to a greater degree as the evidence of maternal vascular damage increases [1 7] Their classification of cases and the schedule of hormone dosage are shown in Tables 3 and 4 White and her associates report 90 per cent fetal survival with the use of female sex hormones but stress that the hormone therapy is but one seventh part of their total treatment program Their total treatment consists of seven parts (1) classification of patients (2) chemical control of diabetes (3) female sex hormone therapy (4) measures used to prevent or correct edema and especially hydramnios (5) early timing of delivery (6) special care of the infant in the immediate postnatal period and (7) yearly observation of the offspring of the diabetic mother for evidence clinical or chemical of the disorder diabetes [1 7] The meticulous and superb *medical and obstetrical care given to this group of* patients by Dr White and her associates may eventually prove to be the reason for their consistently excellent fetal survival rate exclusive of hormone therapy it has long been hoped that Dr White would run a control series without hormonal therapy in an effort to determine once and for all the merits of female sex hormone therapy

It is difficult to compare some of the other diabetic studies with those of Dr White because of classification difficulties and lack of individualization in the management of each case For example the British Medical Research Council [72] found the fetal survival rate to be almost identical about 70 per cent in 76 hormone treated and 71 placebo treated patients however oral estrogen and no progesterone was employed and the data accumulated from nine hospitals assumed to provide equal obstetrical medical and pediatric care most of these cases fall into White s classification of Groups A B and C 84 per cent fetal survival is reported from the Boston Lying in *Hospital clinic* (1945 52) where 67 per cent of the 42 cases treated fell into Groups C through F these patients received oral estrogen (stilbestrol) therapy

Jones [59] observed 184 diabetic pregnancies of which 162 reached viability without hormone therapy although the fetal mortality was 29 6 per cent Hurwitz and Higano [53] had 124 viable pregnancies without hormone therapy in a group of 140 patients Reis *et al* [9] observed 70 pregnancies in 52 diabetic women with 86 per cent fetal salvage The fetal salvage rates regardless *of endocrine or nonendocrine* treatment have improved in those cases reported since 1950 Pedowitz and Shelvin [83] reported the end results in 246 diabetic pregnancies from 1932 to 1953 the average fetal mortality of 17 3 per cent *in* viable fetuses was found to average 22 per cent in cases prior to 1950 but only 8 2 per cent in the

cases between 1950 53 The high fetal salvage of about 90 per cent now reported for endocrine and nonendocrine treated cases could be attributed to more adequate prenatal care with co operative patients and early termination of the pregnancy by induction or section at the 35th 37th week There is general agreement that the incidence of spontaneous abortion and toxemia is about the same in *the diabetic and nondiabetic groups and that the* use of hormones has not led to a decrease in these categories

Aside from female sex hormones there is strong *evidence for an increased production of adrenal* cortical hormones late in normal pregnancy so that *profound changes in the urinary excretion of steroid* hormones and their metabolites will have to be anticipated in pregnant diabetic women These factors are being studied by Davis and Plotz As shown in Figure 5 they found the urinary excretion of androsterone etiocholanolone and 11 oxygenated 17 keto steroids decreased normally until the 29th week of pregnancy and were then found in increasing amounts from the 29th to the 36th week of pregnancy in the urine of diabetic mothers Since these steroid metabolites continued to decrease late in normal pregnancy *they felt that* this abnormality in the excretion pattern of certain 17 ketosteroids indicated a disturbance of the adrenal placental interrelationship which could be explained by a decrease in the hormonal activity of the aging placenta

Our knowledge of the pregnant diabetic is still *superficial Adequate control studies are still needed* to prove or disprove the merit of hormonal therapy *Excellent medical obstetrical and pediatric care* with co operative patients has markedly reduced the *rate of fetal wastage in pregnant diabetic patients* but the problems of spontaneous abortion premature *labor and toxemia in these cases still presents* a challenging field of research for the elimination of fetal loss in diabetics

PREMATURE LABOR

Approximately two thirds of premature infants result from mothers who have spontaneous premature labor and delivery for no known reason although the question of hyperactivity of the uterus or insufficient placental hormones has long been theorized In the course of their study designed to analyze the hormone excretion and uterine motility in the same

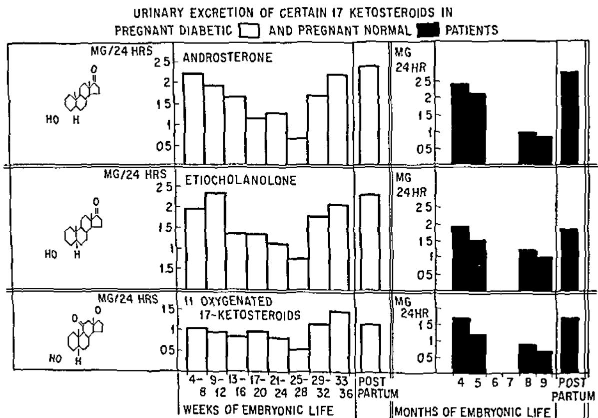


FIG 5 URINARY EXCRETION OF CERTAIN 17 KETOSTEROIDS IN PREGNANT DIABETIC AND PREGNANT NORMAL PATIENTS (Davis and Plotz Obst. & Gynec Survey 1 1 1956)

Table 3 CLASSIFICATION OF DIABETIC PATIENTS AS TO RISK OF PREGNANCY

(White et al Am J Obst & Gynec 71 57 1956)

Class A—Diabetes shown by glucose tolerance test
Class B—Diabetes with onset after age 20 duration 0–9 years and no vascular disease
Class C—Diabetes with onset between ages of 10 and 19 duration 10–19 years and no vascular disease
Class D—Diabetes with onset before age 10 duration 20+ years and vascular disease evidenced by calcification of leg arteries and retinopathy
Class E—Diabetes with calcified pelvic arteries
Class F—Diabetes with nephropathy

Table 4 DAILY INTRAMUSCULAR DOSEAGE OF STILBESTROL AND PROLUTON (IN MG)

(White et al Am J Obst & Gynec 71 57 1956)

Week of pregnancy	Class				
	B	C	D	E	F
0–16	25	25	25	25	25
17–19	25	25	50	50	50
20–23	50	50	100	100	100
24–29	100	100	125	125	125
30–33	100	100	150	150	150
34 to end	125	125	200	200–250	200–250

the Black divisions who have a common objective but no controlling policy All use good maternal and fetal care proper diet insulin and early delivery to reach their objective but differ in organization philosophy and choice of auxiliary weapons [5] The White division [75 127 128] considers the use of estrogen and progesterone very important the Black division [33 53 59 8] does not use hormones and the small Gray division also uses hormones but in other than the recommended dosage [7 84 107]

For years the White division headed by Priscilla White at the Joslin Clinic has advocated and emphasized the need of hormone therapy and has come to five general conclusions First that an imbalance of female sex hormones occurs in abnormal pregnancies in diabetic patients low levels for serum estrogen low levels of pregnanediol excretion and high levels for chorionic gonadotropin have been the common hormonal pattern Secondly that this imbalance predicts or precedes the complications of pregnancy Thirdly that the imbalance can be corrected by the administration of female sex hormones which should be administered by the intramuscular route Fourthly that such correction leads to improved fetal survival provided other parts of management of pregnancy and diabetes are observed And finally that the imbalance occurs more often and to a greater degree as the evidence of maternal vascular damage increases [1 7] Their classification of cases and the schedule of hormone dosage are shown in Tables 3 and 4 White and her associates report 90 per cent fetal survival with the use of female sex hormones but stress that the hormone therapy is but one seventh part of their total treatment program Their total treatment consists of seven parts (1) classification of patients (2) chemical control of diabetes (3) female sex hormone therapy (4) measures used to prevent or correct edema and especially hydramnios (5) early timing of delivery (6) special care of the infant in the immediate postnatal period and (7) yearly observation of the offspring of the diabetic mother for evidence clinical or chemical of the disorder diabetes [127] The meticulous and superb medical and obstetrical care given to this group of patients by Dr White and her associates may eventually prove to be the reason for their consistently excellent fetal survival rate exclusive of hormone therapy it has long been hoped that Dr White would run a control series without hormonal therapy in an effort to determine once and for all the merits of female sex hormone therapy

It is difficult to compare some of the other diabetic studies with those of Dr White because of classification difficulties and lack of individualization in the management of each case For example the British Medical Research Council [72] found the fetal survival rate to be almost identical about 70 per cent in 76 hormone treated and 71 placebo treated patients however oral estrogen and no progesterone was employed and the data accumulated from nine hospitals assumed to provide equal obstetrical medical and pediatric care most of these cases fall into White s classification of Groups A B and C 84 per cent fetal survival is reported from the Boston Lying in Hospital clinic (1945 52) where 67 per cent of the 42 cases treated fell into Groups C through F these patients received oral estrogen (stilbestrol) therapy

Jones [59] observed 184 diabetic pregnancies of which 162 reached viability without hormone therapy although the fetal mortality was 29 6 per cent Hurwitz and Higano [53] had 124 viable pregnancies without hormone therapy in a group of 140 patients Reis *et al* [9] observed 70 pregnancies in 52 diabetic women with 86 per cent fetal salvage The fetal salvage rates regardless of endocrine or nonendocrine treatment have improved in those cases reported since 1950 Pedowitz and Shlevin [83] reported the end results in 246 diabetic pregnancies from 1932 to 1953 the average fetal mortality of 17 3 per cent in viable fetuses was found to average 22 per cent in cases prior to 1950 but only 8 2 per cent in the

with very low HCG titers have been described[90] Even the appearance of HCG in spinal fluid does not seem too conclusive although some workers are impressed with the significance of HCG concentration in spinal fluid in the diagnosis of moles and chorionepitheliomas[18 71] It would seem that the diagnosis of hydatidiform mole should be based mainly on clinical signs treatment consists of total evacuation of the uterine contents While the HCG level may or may not aid in the diagnosis of a mole it is invaluable in the follow up study of these cases since about 5 per cent of moles will undergo malignant degeneration into chorionepitheliomas A few cases of repeated hydatidiform mole have been reported[66]

Chorionepithelioma is a highly malignant tumor arising from the chorionic epithelium which may follow evacuation of a mole an abortion or a normal pregnancy Park and Lee reported 50 per cent of these choriocarcinomas followed a hydatid mole 30 per cent followed abortion and 20 per cent followed a normal pregnancy[78] Payne and Seigler pointed out that a negative standard gonadotropin pregnancy test following any of these conditions with the subsequent development of a positive test was strongly suggestive of chorionepithelioma provided a new pregnancy could be ruled out[0 95] Following the evacuation of a mole HCG assays should be made at regular intervals of two weeks for the first two or three months and subsequently checked at longer intervals until a year has passed Various follow up programs are pursued but in general it is necessary to establish an early base line level of HCG thereafter a rising titer of HCG regardless of the absolute initial level is strong evidence for the presence of malignant tissue growth if a new pregnancy can be excluded Radical surgery is justified in the treatment of this disease even if metastases are known to be present since removal of the local tumor has been followed by the regression of the metastatic growth in some cases

SHEEHAN S SYNDROME

The development of anterior pituitary deficiency in women who survived postpartum hemorrhage and/or shock has been described as Sheehan s syndrome[1] During the resultant general circulatory disturbance the anterior lobe of the pituitary deprived of its blood supply undergoes ischemic necrosis If the original necrosis involves about two-thirds to three fourths of the lobe there will be some subsequent pituitary insufficiency and if practically all the lobe is involved as it has been in about one half of all the observed cases the pituitary insufficiency will be very severe Sheehan and Murdock[10] followed a group of obstetrical patients who had some degree of hemorrhage and/or shock at the time of delivery and found that 15 per cent of the survivors from moderate hemorrhage or shock and 40 per cent of the survivors from severe hemorrhage or shock had subsequent hypopituitarism They feel that the incidence of this syndrome is even higher since in many instances the symptoms occur so long after the pregnancy that the pituitary insufficiency is wrongly attributed to Simmonds disease and others also feel that this condition has frequently gone unrecognized[54] Future studies in cases of moderate to severe postpartum hemorrhage and/or shock are designed to estimate the endocrine functions of the anterior pituitary gland the thyroid adrenals and ovaries Periodic bioassays of 24 hour urines for gonadotropins estrogen 17 ketosteroids and formaldehydogenic corticoids should be done radioactive iodine uptake serum protein bound iodine and serum cholesterol studies will be useful as well as the insulin tolerance test eosinophile counts and the Kepler water excretion tests Using these studies on a group of 8 patients with 6 normal post partum cases as controls Schneeberg and his associates[94] found 3 patients who showed various degrees of anterior pituitary hypofunction When the 8 patients were compared as a group to the 6 control women their subnormal output of 17 ketosteroids was evident (range 2 7 14 3 mgm for the patients and 8 14 9 for the controls) in all the other tests there was no significance between the two groups It would seem worthwhile on the basis of the accumulated cases that all survivors of moderate to severe postpartum hemorrhage and shock should be studied at repeated intervals for evidences of pituitary insufficiency

ADRENAL HEMORRHAGE IN PREGNANCY

About 35 cases of adrenal hemorrhage associated with pregnancy have been recorded all but two occurred during a pathological gestation All cases were diagnosed at autopsy and apparently the true condition was not suspected In terms of the stress theory of adaptation pregnancy itself requires an adaptation to stress and any complication of the pregnancy may lead to the exhaustion reaction of Selye[89] in the form of acute adrenal insufficiency The diagnosis when considered can be confirmed by the eosinophil test which will show no decrease in eosinophils when ACTH is injected Intravenous administration of 5 100 cc of aqueous adrenal cortical extract will alleviate the symptoms quite dramatically Cortisone and desoxycortico terone are not as rapid in their action or over all effect as the aqueous cortical extracts and the danger of inadequate dosage or overdosage is great Administra

patients from early pregnancy to delivery Taylor and his associate[118] observed 4 patients who had spontaneous premature delivery at 35 36 weeks gestation study of the uterine motility recordings of these patients disclosed that as early as the 26th week their uteri were excessively irritable there was suggestive evidence of a relatively low excretion of total estrogens associated with premature delivery pregnanediol values did not vary with uterine motility and apparently had no relationship to the premature onset of labor

About half of the cases admitted to the hospital with premature labor will cease with bed rest sedation and antibiotics Various hormone regimens of estrogen progesterone or both have been used ineffectively Eichner *et al*[30] reported on 60 patients with premature spontaneous rupture of the membranes between the 20th and 30th weeks of gestation 18 of which were controls and the remainder treated with massive doses of progesterone 200 600 mgm I M daily progesterone did seem to lengthen slightly the latent period between rupture of the membranes and the onset of labor

Attention is now chiefly centered on the use of relaxin a nonsteroid hormone of protein nature identified from corpus luteum extracts by Hisaw in 1929 who showed this substance was responsible for the relaxation of the pelvic ligaments in pregnancy[50] Since then work with this substance has proceeded irregularly since species specificity apparently modifies the effects of the hormone Currently the ovary of the sow is the source of the most potent material in spite of the fact that it is a protein no evidence of antigenicity or toxicity has been found even with the administration of very large doses Relaxin (Releasin Warner Chilcott) was administered to 78 patients by the intravenous and intramuscular routes in an experimental study and its use was recommended when premature labor begins between the 29th and 36th weeks and before cervical dilatation has progressed beyond 3 cm[31] softening of the cervix was noted with the use of relaxin but this did not necessarily lead to effacement nor was there evidence that it increased the success of induction labor was of short duration and often precipitous after administration of the hormone Folsome *et al*[34] have reported on the use of relaxin in 40 cases of premature labor with 50 cases as a control group The control group was treated with bed rest sedation and high protein high vitamin diet only seven infants (14%) were salvaged from this group Administration of relaxin to 40 patients with premature labor resulted in a sufficient gain of *in utero* time to allow delivery of 27 normal infants (67 5%) which is a substantial decrease in fetal wastage Abramson and Reid[1] had five cases of premature labor between the 29th and 31st week of gestation labor ceased following the *intramuscular* injection of relaxin and pregnancy continued through the 36th to 40th weeks *with delivery of normal* infants

HYDATIDIFORM MOLE AND CHORIONEPITHELIOMA

Hydatidiform mole is a condition due to proliferative degeneration of the chorionic villi resulting in hydropic or grape like swelling to the villous branches In a sense it is the temporary missed abortion of a blighted ovum in which the stroma of the chorionic villi becomes edematous in the presence of functioning trophoblast It occurs about once in 2000 deliveries (Boston Lying in Hospital incidence) The condition is suspected usually near the end of the first trimester of pregnancy when a disproportionately large uterus is felt though Mathieu[70] has emphasized much variability in this sign Vaginal bleeding nearly always occurs though uterine cramps are usually absent Furthermore no fetal heart tones are heard nor is any fetal skeleton visualized by x ray The passage of typical grape like structures gives the obvious diagnosis but this seldom happens The usual time for delivery of a mole is 17 18 weeks

Since hydatidiform mole is associated with trophoblastic proliferation increased hormonal production could be expected The prevalent ideas that estrogen and progesterone production were markedly diminished or even absent have been disproved Estrogen secretion within the normal range has been reported[49 80] At first it was thought that the measurement of urinary pregnanediol would be low or absent in hydatidiform mole and chorionepithelioma therefore serving to differentiate these conditions from normal pregnancy but de Watteville[1 5] reported three cases of hydatid mole in which pregnanediol was present Similar findings of pregnanediol have been reported thus rendering this differential point less secure

There is usually an increased production of chorionic gonadotropin in hydatid mole but a high titer in blood or urine is not always of diagnostic aid Since high HCG levels may still be present at the end of the first trimester when a suspicion of mole may arise the diagnosis of a mole should not be made on the basis of a single HCG titer although levels of more than 2 500 000 mouse units per 24 hours in the urine or more than 100 000 units per 100 ml in the serum should be regarded with suspicion[79] Multiple pregnancy hyperemesis gravidarum and even normal pregnancy have been associated with high HCG levels Several workers have found the range in HCG excretion to overlap considerably with normal pregnancy levels and exceptional cases

cotropic substances from human placenta Endocrinology 55 561 7
7 Baker W S Bancroft C E Lyda E W and Lehman J J 1955 The value of urinary pregnanediol determinations as an indication for the use of progesterone in the treatment of threatened abortion Am J Obst & Gynec 69 405-14
8 Barnes A C and Quilligan E J 1956 Measurement of aldosterone *in the eclamptogenic toxemias* of pregnancy Am J Obst & Gynec 71 670 82
9 Beclere C and Jayle M 1952 Hormonal dosage in pregnant women with previous pathological pregnancies Compte rend Soc franc gynec 22 117-32
10 Behrman S J and Niemann P 1955 Serum gonadotropins in normal and abnormal pregnancy Fert & Steril 6 236-46
11 Berle B B and Javert C T 1954 Stress and habitual abortion their relationship and the effect of therapy Obst & Gynec 3 298 306
12 Bevis D C A 1951 Treatment of habitual abortion Lancet 2 207
13 Bishop P M F and Richards N A 1952 Habitual abortion further observations on prophylactic value of progesterone pellet implantation Brit M J 1 244 6
14 Borth R and Watteville H de 1952 Hormone assays in obstetrics and gynecology In Vitamins and Hormones New York Acad Press 10 141 75
15 Bradbury J T Brown W E and Gray L A 1949 Maintenance of corpus luteum and physiologic actions of progesterone In Recent Progress in Hormone Research New York Acad Press 5 151 96
16 Brody H and Horowitz E A 1950 Gonadotropin in spinal fluid in a case of twin pregnancy criticism of spinal fluid test for diagnosis of hydatidiform mole Am J Obst & Gynec 59 685-8
17 Brown W E and Bradbury J T 1947 Study of physiologic action of human chorionic hormone Am J Obst & Gynec 53 749 57
18 Carreras B B 1954 Intravenous ACTH in the treatment of hyperemesis gravidarum Obst & Gynec 3 50 52
19 Chart J J Shipley E G and Gordon E S 1951 Evidence for a sodium retaining factor in toxemia of pregnancy Proc Soc Exper Biol & Med 78 244 6
20 Colman E D Bartholomew R A Grimes W H and Fish J S 1950 Salvage possibilities in threatened abortion Am J Obst & Gynec 59 1208 24
21 Comminos A C 1956 Thyroid function and therapy in reproductive disturbances Obst & Gynec 7 260 67
22 Crowder R E Bills E S and Broadbent J S 1950 The management of threatened abortions a study of 100 cases Am J Obst & Gynec 60 896 9
23 Davis M E and Fugo N W 1948 Does administration of diethylstilbestrol to pregnant women result in increased output of urinary pregnanediol? Proc Soc Exper Biol & Med 69 436 8
24 Davis M E and Plotz E J 1956 Hormonal interrelationships between maternal adrenal placental and fetal adrenal function OB GYN Survey 11 1-43
25 DeCosta E J 1955 Diabetes and pregnancy Obst & Gynec 5 401 9
26 Diddle A W O Connor K A Jack R and Pearse R L 1953 Evaluation of bed rest in threatened abortion Obst & Gynec 2 63 7
27 Dieckmann W J 1952 In Toxemias of Pregnancy chaps IX X XI ed 2 St Louis The C V Mosby Company pp 250 302
28 Dieckmann W J Davis M E Rynkiewicz L M and Pottinger R E 1953 Does the administration of diethylstilbestrol during pregnancy have therapeutic value? Am J Obst & Gynec 66 1062 81
29 Eastman N J 1946 Habitual abortion In Progress in Gynecology ed 2 Grune and Stratton New York pp 262 8
30 Eichner E Kunin K Linden M Goldberg I Salinger L and Peller Z 1954 The use of large doses of progesterone in delaying the onset of labor after premature spontaneous rupture of the membranes Am J Obst & Gynec 67 339 41
31 Eichner E Waltner C Goodman M and Post S 1956 Relaxin the third ovarian hormone its experimental use in women Am J Obst & Gynec 71 1035-48
32 Ferguson J H 1953 *Effect of stilbestrol on pregnancy* compared to the effect of a placebo Am J Obst & Gynec 65 592-601
33 Flowers F C and Levitt L M 1954 The effect of improved medical and obstetrical care upon fetal and maternal mortality in pregnant diabetics South Med J 47 215 21
34 Folsome C E Harami T Lavietes S R and Massell G M 1956 A clinical evaluation of relaxin in premature labor premature ruptured membranes and full term obstetric cases with suspected small babies Obst & Gynec 8 536-44
35 Forbes A P Henneman P H Griswold G C and Albright F 1954 Syndrome characterized by galactorrhea amenorrhea and low urinary FSH J Clin Endocrinol & Metab 14 265 71
36 Fried P H and Rakoff A E 1952 Effects of chorionic gonadotropin and prolactin on maintenance of corpus luteum function J Clin Endocrinol 12 321 37
37 Gordon E S Chart J J Hagedon D and Shipley E G 1954 Mechanism of sodium retention in pre eclamptic toxemia Obst & Gynec 4 39 50
38 Greenblatt R B Carmona N and Hagler W S 1956 Chiari Frommel syndrome Obst & Gynec 7 165 70
39 Guterman H S 1946 Prediction of fate of threatened abortion by pregnanediol J A M A 131 378 82
40 ——— 1950 The diagnostic significance of pregnanediol excretion In Progress in Clinical Endocrinology New York Grune and Stratton pp 422 8
41 ——— 1953 Progesterone metabolism in the human female its significance to reproduction In Recent Progress in Hormone Research New York Acad Press 8 293-332
42 Guterman H S and Schroeder M S 1948 A simplified technique for the quantitative estimation of pregnanediol in urine J Lab & Clin Med 33 356 66
43 Guterman H S and Tulsky A S 1949 Observations on use of progesterone in threatened abortion with special reference to pregnanediol excretion as a guide to therapy Am J Obst & Gynec 58 495-502
44 Ham G C and Landis E M 1942 Comparison of pituitrin with antidiuretic substance found in human urine and placenta J Clin Investigation 21 455-70
45 Hamblen E C 1941 Some clinical observations on the endocrinology of abortion Am J Obst & Gynec 41 644 75

tion of glucose saline ascorbic acid thiamine and pyridoxine potentiates the effect of ACTH [5]

DISTURBANCES IN LACTATION

Breast tissue capable of responding normally to estrogens progesterone and lactogenic hormone is essential for successful lactation It is also evident that the mechanical stimulus of milking or suckling the breast is as important in initiating and maintaining lactation as is the proper hormonal stimulation of the breast Failure of lactation is primarily due to inadequate suckling or failure to continue nursing although it may be due to genetically inadequate breast tissue or inadequate secretion of lactogenic hormone The administration of prolactin intramuscularly is sometimes helpful in stimulating the flow of milk

Much has been written about the use of estrogens and androgens for suppression of lactation and prevention of painful breast engorgement The general conclusion that diethylstilbestrol does not reduce the milk supply or prevent the establishment of lactation where nursing is permitted has been confirmed [4] primary engorgement of the breasts may possibly be avoided by the use of estrogen Estrogens have not totally relieved the painful breast engorgement occurring in nonnursing mothers or in mothers who wish to give up nursing poor involution or withdrawal bleeding has not been a problem in most cases treated with estrogens but occasionally recurrence of the engorgement follows cessation of the hormone Testosterone and depo testosterone have also been fairly satisfactory in relieving painful breast engorgement Since the response to either hormone is largely an individual affair and since the transient symptoms of breast engorgement rarely persist longer than 48 hours the routine use of hormones in the nonnursing mother is not recommended

The syndrome of amenorrhea and lactation persisting in otherwise normal postpartum women was first described by Chiari in 1832 and later elaborated on by Frommel in 1882 This condition now known as the Chiari Frommel syndrome is still not clearly understood as to its physiopathology The high incidence of pituitary tumors in these cases suggests that the syndrome is due to pituitary dysfunction from a pituitary chromophobe adenoma that permits the production of an excessive amount of prolactin [35] though Argonz and del Castillo [4] feel the syndrome results in increased prolactin secretion due to hyperfunction of the eosinophilic cells of the anterior lobe of the pituitary Endocrine studies consistently show low to absent FSH decreased excretion of estrogens and normal urinary 17 ketosteroids Protein bound iodine levels are usually below normal Urinary pregnanediol excretion is not significant This syndrome is markedly resistant to all kinds of therapy estrogen progesterone estrogen progesterone androgens thyroid chorionic gonadotropin x ray and surgery have been tried with equivocal results [35] large doses of cyclic estrogen progesterone therapy have yielded some improvement so far hydrocortisone has been of no value

CONCLUSIONS

The status of hormones and hormonal therapy in the disturbances of pregnancy may be compared to a difficult and intricate jig saw puzzle Certain portions of this hormone puzzle fit together well and give a clear concept of that aspect of the problem other portions are still incomplete and largely theoretical Certain fitted portions have to be reassembled as new investigative procedures are evolved and clinical evidence is tabulated larger series of cases adequate control groups and more reliable uniformly standardized laboratory tests will eventually give the necessary information regarding the use and abuse of hormonal therapy As background pieces in this puzzle are etiological factors for abortion toxemia premature labor and diabetes which are as yet unknown and without which the puzzle cannot be totally completed hormone imbalances appear to be the result and not the cause of pregnancy disturbances Many investigators will continue to work toward the completion of this puzzle but all must use the same identical pieces regarding choice of procedures and patients treatment of adequate control groups and standardized general care of the patient irrespective of geographical location or type of practice It is only in this way that enough data can be accumulated so that statistically *valid conclusions* can be drawn regarding the role of hormones and hormonal therapy in the disturbances of pregnancy As to the hormone future one should recall that the sky is usually darkest before the dawn

BIBLIOGRAPHY

1 Abramson D and Reid D E 1955 Use of relaxin in threatened premature labor J Clin Endocrinol & Metab 15 206 9
2 Agnew A M Palmer R A Bagnall A W and McIntosh H W 1953 Cortisone in the treatment of toxemia of pregnancy Obst & Gynec 1 282 6
3 Anselmino K J Hoffman F and Kennedy W P 1932 Relation of hyperfunction of posterior lobe of hypophysis to eclampsia and neuropathy of pregnancy Edinburgh M J 39 376 88
4 Argonz J and del Castillo E B 1953 A syndrome characterized by estrogenic insufficiency galactorrhea and decreased urinary gonadotropin J Clin Endocrinol & Metab 13 79 87
5 Assali N S Clark L C Jr and Sobel E H 1953 Studies of toxemia of pregnancy J Clin Endocrinol 13 1030-42
6 Assali N S and Hamermesz J 1954 *Adrenocorti*

93 *Rosenblum* G and Melinhoff E 1947 Preservation of threatened pregnancy with particular reference to use of diethylstilbestrol West J Surg 55 597-603
94 Rucker M P 1952 Spontaneous abortions too many or too few? J Internat Coll. Surgeons 17 328-51
95 *Schoeneck* F J 1942 Gonadotropic hormone concentration in emesis gravidarum Am J Obst & Gynec 43 308 12
96 Schneeberg N G Perloff W H Viellard C B and Israel S L 1953 An endocrine study of the survivors of postpartum hemorrhage and shock Obst & Gynec 1 156-64
97 Segaloff A Sternberg W H and Gaskill, C J 1951 Effects of luteotropic doses of chorionic gonadotropin in women J Clin Endocrinol 11 936-44
98 Seigler S L David J M and Tobin S M 1950 An appraisal chorionepithelioma based on observations in 12 cases Am J Obst. & Gynec 60 586-604
99 Selye H 1946 General adaptation syndrome and diseases of adaptation J Clin Endocrinol 6 117 230
100 Sheehan H L 1937 Post partum necrosis of anterior pituitary J Path & Bact 45 189 214
101 Sheehan H L and Murdoch R 1938 Post partum necrosis of anterior pituitary pathological and clinical aspects J Obst & Gynaec Brit Emp 45 456 89
102 Smith G V 1950 In Textbook of Endocrinology Williams R H Philadelphia W B Saunders Co pp 349-449
103 Smith G V and Smith O W 1935 Further quantitative determinations of prolan and estrin in pregnancy Surg Gynec & Obst. 61 27 35
104 —— 1940 Estrogen and progestin metabolism in pregnant women Am J Obst & Gynec 39 405-22
105 —— 1941 Estrogen and progestin metabolism in pregnancy the endocrine imbalance of pre-eclampsia and eclampsia summary of findings to February 1941 J Clin Endocrinol 1 470-76
106 —— 1948 Internal secretions and toxemia of late pregnancy Physiol Rev 28 1 22
107 —— 1954 Prophylactic hormone therapy Obst & Gynec 4 129-41
108 Smith O W 1948 Diethylstilbestrol in the prevention and treatment of complications of pregnancy Am J Obst & Gynec 56 821-34
109 —— 1950 The quantitative determination of urinary pregnanediol in pregnant women the influence of the method upon results J Clin Endocrinol 10 490-510
110 Smith O W and Barker C 1952 Factors influencing the composition of the sodium pregnanediol glucuronide complex in human pregnancy with special reference to diethylstilbestrol therapy J Clin Endocrinol 12 151-68
111 Smith O W and Schiller S 1950 Does the excretion of diethylstilbestrol glucuronide influence the Venning determination of urinary pregnanediol? Proc Soc Exper Biol & Med 73 378 81
112 Smith O W and Smith, G V 1949 The influence of diethylstilbestrol on the progress and outcome of pregnancy as based on a comparison of treated and untreated primigravidas Am J Obst & Gynec 58 994 1009
113 Sommerville I F Gough, N and Marrian G F 1948 The quantitative determination of small amounts of pregnanediol in human urine J Endocrinol 5 247 57
114 Sommerville I F Marrian C F and Clayton B E 1949 Effect of diethylstilbestrol on urinary excretion of pregnanediol and endogenous estrogens Lancet 1 680-82
115 Speert H 1954 Pregnancy prognosis following repeated abortions Am J Obst. & Gynec 68 665-73
116 Sulman F G and Bergman F 1953 Adrenocorticotropic activity of placental extracts J Obst & Gynaec Brit Emp 60 123-5
117 Swyer C I M and Daley D 1953 Progesterone implantation in habitual abortion Brit M J 1 1073-7
118 Taylor E S Bruns P D Anker R M and Drose V E 1955 Correlation of urinary estrogen pregnanediol excretion with uterine motility during pregnancy Am J Obst & Gynec 70 894 909
119 Tew W P and McAlpine H T 1950 Eclampsia treated with cortisone Canad M A J 63 287
120 Tobian L 1949 Cortical steroid excretion in edema of pregnancy pre-eclampsia and eclampsia J Clin Endocrinol 9 319 29
121 Tolstoi E Given W P and Douglas R G 1953 Management of the pregnant diabetic J A M A 153 998-1002
122 Vanden Bosch M P 1951 ACTH in the treatment of hyperemesis gravidarum Am J Obst & Gynec 62 456 7
123 Venning E H 1937 Gravimetric method for the determination of sodium pregnanediol glucuronide J Biol Chem 119 473-80
124 Venning E H Singer B and Simpson G A 1954 Adrenocortical function in toxemia of pregnancy Am J Obst & Gynec 67 542 8
125 Watteville H de 1951 Pregnanediol determinations in clinic and in research J Clin Endocrinol 11 251-66
126 Wells C N 1953 Treatment of hyperemesis gravidarum with cortisone Am J Obst & Gynec 66 598-601
127 White P 1949 Pregnancy complicating diabetes Am J Med 7 609 16
128 White P Gillespie L and Sexton L 1956 Use of female sex hormone therapy in pregnant diabetic patients Am J Obst & Gynec 71 57-69
129 Willson R J Carrington E R Hadd H F and Bontwell J 1954 Pregnancy edema Obst & Gynec 3 651-61
130 Wilson, R B 1955 Habitual abortion hormonal physiology and a suggested endocrine treatment for selected patients Am J Obst & Gynec 69 614 28
131 Zondek B 1954 Some problems related to ovarian function and to pregnancy In Proc Laurentian Hormone Conference 10 395-416
132 Zondek B Forman I and Cooper K L 1955 Placental insufficiency cervical mucus arborization as aid in diagnosis Fert & Steril 6 5-3-33
133 Zondek B Sulman F and Black R 1948 Hormonal test for fetal death in disturbed pregnancy J A M A 136 965-9
134 Youseff A F and Barsoum G S 1953 Vomiting of pregnancy Allergic factor J Obst & Gynaec Brit Emp 60 398-97

46 Harvey W A and Sherfey M J 1954 Vomiting in pregnancy a psychiatric study Psychosom Med 16 1 9
47 Hertig A G and Edmonds H E 1940 Genesis of hydatidiform mole Arch Path 30 260 91
48 Hesseltine H C Bustamante J and Navori C A 1955 Limited influence of diethylstilbestrol on lactation *Am J Obst & Gynec* 69 686 92
49 Hinglais H and Hinglais M 1949 Etude hormonale de la mole hydatidiforme Compt rend Soc de biol 143 321 4
50 Hisaw F L 1929 Corpus luteum hormone experimental relaxation of pelvic ligaments of guinea pig Physiol Zool 2 59 79
51 Hughes E C Lloyd C W Jones D Lobotsky J Rienzo J S and Avery G H 1954 Some recent observations concerning the toxemias of pregnancy Am J Obst & Gynec 67 782 800
52 Hughes E C VanNess A W and Lloyd C W 1950 The nutritional value of the endometrium for implantation and in habitual abortion Am J Obst & Gynec 59 1292 1303
53 Hurwitz D and Higano N 1952 Diabetes and *pregnancy* New Eng J Med 247 305 9
54 Israel S L and Conston A S 1952 Unrecognized pituitary necrosis (Sheehan s syndrome) J A M A 148 189 93
55 Javert C T 1954 Repeated abortion results of treatment in 100 patients Obst & Gynec 3 420 34
56 Javert C T and Finn W F 1950 Observations on pathology of spontaneous abortion preliminary report of 500 cases Texas State J Med 46 739-45
57 Jayle M F 1952 Elimination des steroids au cours de la gestation normale ou pathologique Compt rend Soc franc gynec 12 405
58 Jones G E S and Delfs E 1951 Endocrine patterns in term pregnancies following abortion J A M A 146 1212 18
59 Jones W S 1953 Diabetes in pregnancy Am J Obst & Gynec 66 322-34
60 King A G 1953 Threatened and repeated abortion present status of therapy Obst & Gynec 1 104 14
61 Kinnunen O and Niemeva K 1950 The foetal adrenals in maternal toxemias Acta Endocrinol 5 165 72
62 Koff A K and Tulsky A S 1953 Threatened abortion evaluation of prognosis based on pregnanediol determinations and of treatment with progesterone S Clin North America 33 3 16
63 Kotz J Parker E and Kaufman M S 1941 Treatment of recurrent and threatened abortion report of 226 cases J Clin Endocrinol 1 838-49
64 Long W N Hartmann W L Futcher P H and Eastman N J 1954 Diabetes mellitus and pregnancy Obst & Gynec 3 160 68
65 Malphas P 1938 A study of abortion sequences J Obst & Gynaec Brit Emp 45 932 49
66 Mann E C 1956 Psychiatric investigation of habitual abortion preliminary report Obst & Gynec 7 589 601
67 Margulis R R and Hodgkinson C P 1953 Evaluation of safety of corticotropin (ACTH) and cortisone in pregnancy Obst & Gynec 1 276 81
68 Margulis R R Hodgkinson C P Rattan W C and Jewell J S 1954 Toxemia of pregnancy—ACTH and cortisone therapy Am J Obst & Gynec 67 1237-48
69 Mastboom J L 1952 The importance of adreno-cortical steroids for the development of toxaemia of late pregnancy Gynaecologia 134 217 43
70 Mathieu A 1939 Hydatidiform mole and chorion epithelioma Surg Gynec & Obst 68 844 5
71 McCormick J B 1954 Gonadotropin in urine and spinal fluid Obst & Gynec 3 58-66
72 Medical Research Council Conference on Diabetes *and Pregnancy 1955 Use* of hormones in management of pregnancy in diabetes Lancet 2 833 6
73 Moore H Jessop W J E O Donovan D K Barry A P Quinn B and Drury M I 1951 Cortisone in the treatment of toxaemia of pregnancy Brit M J 1 841 50
74 Mukherjee C 1941 Posterior pituitary factor in toxaemias of pregnancy J Obst & Gynaec Brit Emp 48 586 609
75 Nelson H B Gillespie L and White P 1953 Pregnancy complicating diabetes mellitus Obst & Gynec 1 219 25
76 Opsahl J C and Long C N H 1951 Identification of ACTH in human placental tissue Yale J Biol & Med 24 199 209
77 Papanicolaou G N 1946 General survey of vag*inal smear and its use in research and diagnosis* Am J Obst & Gynec 51 316-28
78 Park W W and Lees J C 1950 Choriocarcinoma a general review with an analysis of 516 cases Arch Path 49 73 104
79 Paschkis K E Rakoff A E and Cantarow A 1954 Clinical Endocrinology Chap 21 New York Paul B Hoeber Inc pp 489 520
80 Payne F L 1941 Hormone studies in presence of hydatidiform mole and chorionepithelioma Surg Gynec & Obst 73 86-95
81 Pearlman W H Pearlman M R J and Rakoff A E 1953 Estrogen metabolism in human pregnancy Am J Obst & Gynec 66 370 74
82 Pedowitz Paul and Shelvin E L 1952 The pregnant diabetic patient Bull New York Acad Med 28 440 53
83 —— 1955 The pregnant diabetic patient Am J Obst & Gynec 69 395-404
84 Peel J H 1955 Management of pregnant diabetic Brit M J 2 870 74
85 Peterson W F and Goldzieher J 1953 Adrenal hemorrhage in pregnancy Am J Obst & Gynec 66 648 53
86 Posner A C Kushner J I and Posner L B 1955 Repeated hydatidiform mole Obst & Gynec 5 761 4
87 Randall C L Baetz R W Hall D W and Birtch P K 1955 Pregnancies observed in the likely to abort patient with or without hormone therapy before or after conception Am J Obst & Gynec 69 643 56
88 Randall C L and Birtch P K 1952 Spontaneous abortion diagnosis and treatment Canad M A J 66 132 7
89 Reis R DeCosta E J and Allweiss N 1950 The management of the pregnant diabetic woman and her newborn infant Am J Obst & Gynec 60 1023-42
90 Riehl R 1950 Sur un cas de choriocpithelìome avec réaction de grossesse paradoxale après mold hydatidiforme Gynec et Obst 49 299 306
91 Robinson D and Shettles L B 1952 The use of diethyl tilbestrol in threatened abortion Am J Obst & Gynec 63 1330-33
92 Rogers J 1955 Clinical significance of pregnanediol excretion Fert & Steril 6 513 22

development and delayed menarche is difficult to establish

In many instances retarded development of the accessory sex characteristics is associated with delayed appearance of the menarche However delayed menarche is not infrequently noted in adolescent girls with normally developed secondary sex characteristics

The vast majority of patients with delayed adolescent development manifest no demonstrable organic pathology The developmental retardation in these individuals represents a normal variation in endocrine function which will usually be rectified by time alone For this reason a most perplexing problem with such patients is *when* to commence a diagnostic program The answer lies in the individualization of each case taking into account historical facts physical examination findings and the emotional attitudes of the patient and her family However the practice of initiating therapy to promote sexual development is to be deplored unless adequate diagnostic studies have been undertaken

The endocrine defect in delayed adolescent development is the lack of proper estrogenic stimulation necessary for maturation of the accessory sex organs and initiation of uterine bleeding The secretion of estrogen is dependent upon the elaboration of gonadotropic hormones from the adenohypophysis The secretion of gonadotropins may be diminished or entirely lacking due to lesions of the hypothalamus and the pituitary gland Pituitary adenomas craniopharyngiomas infection and infarction are responsible for the failure of steroid elaboration from the ovaries Malnutrition due to starvation or chronic wasting diseases may also result in hypofunction of the pituitary gland

Delayed adolescent development and delayed menarche result from agenesis hypoplasia or dysfunction of the ovaries Congenital anomalies of the ovaries are discussed in another section of this chapter

Imbalances in other endocrine organs such as the thyroid or the adrenal glands may also delay adolescent development and the menarche Emotional and psychogenic factors may be etiological in certain instances of delayed adolescent development and delayed menarche

Clinical evaluation of patients manifesting delayed adolescent development should commence with a complete and thorough history and physical examination Careful examination of the accessory sex organs and the genitalia is mandatory Examination of the internal genitalia is accomplished by means of rectal examination negligible psychic trauma and physical discomfort ensue if the examiner is reassuring and careful Roentenographic study of the skull for evidence of intracranial lesions is imperative in all cases of retarded development Neurological and ophthalmological examinations are indicated if suspicious signs or symptoms are manifested

Quantitative bioassay of the adenohypophyseal gonadotropins in the urine may be helpful in distinguishing between hypophyseal and ovarian failure An elevated urinary gonadotropic titer suggests ovarian failure whereas diminished titers focus attention on hypophyseal dysfunction Determination of the concentration of estrogens in body fluids would provide the investigator and the physician with valuable data However the quantification of estrogens in blood and urine is impractical due to the gross inaccuracy of existing methodology Some knowledge of ovarian activity is gained from vaginal smears Satisfactory smears are readily obtained from the vaginal tract by means of a glass pipette and rubber suction bulb The cytological characteristics of the vaginal cells reflect the degree of ovarian activity

As previously stated the majority of patients with delayed adolescent development manifest no discernible pathology The prognosis for normal sexual development and normal reproductive function is excellent In the absence of proved lesions and hormonal deficiencies the employment of endocrine preparations is deemed inadvisable Reassurance explanation and time comprise the desired therapeutic regime Systemic abnormalities such as obesity cachexia infection and hypothyroidism should be appropriately treated Adequate psychiatric therapy if indicated should be initiated

The prognosis with proved hypothalamic and hypophyseal lesions is guarded and the assurance of normal pituitary ovarian function cannot be given Maturation of the accessory sex organs and initiation of uterine bleeding in these patients is accomplished with substitutional estrogenic therapy Diethylstilbestrol 5 mgm orally each day or ethinyl estradiol 0 05 mgm orally twice daily is administered for 24 consecutive days at which time the medication is interrupted Withdrawal uterine bleeding usually occurs 2 8 days after cessation of the treatment Diethylstilbestrol or ethinyl estradiol therapy is commenced again on the fourth day following the onset of uterine bleeding and is continued through the twenty fourth day of the artificial cycle Treatment is again interrupted with the appearance of uterine bleeding in 2 8 days These artificial cycles are continued indefinitely Once maturation of the secondary sex characteristics has been effected substitutional therapy can often be omitted entirely unless uterine bleeding is desired by the patient for psychological reasons

Primary ovarian failure is a permanent disability and necessitates substitutional estrogenic therapy as described above for an indefinite length of time Treatment of primary ovarian failure is directed

VII

Gynecologic Disorders

ROBERT W BROWN, M D
AND
ROBERT W KISTNER, M D

DELAYED ADOLESCENT DEVELOPMENT AND AMENORRHEA

Introduction

CONSIDERATION is given in this chapter to certain of the endocrine disorders affecting the female reproductive system Knowledge of the pituitary ovarian and endometrial interactions is essential to an understanding of gynecologic endocrinopathies

The follicle stimulating hormone (FSH) and the luteinizing hormone (LH) are secreted by the adenohypophysis at varying rates throughout the menstrual cycle of the human female The relationship of prolactin to the menstrual cycle remains obscure[8] Although definitive evidence is lacking the assumption that prolactin maintains the corpus luteum in a secretory state continues to prevail Caution should be exercised in ascribing to prolactin this luteotropic role in the human female until satisfactory methods have been developed which will permit positive identification and accurate quantification of prolactin in body tissues and fluids Until such time as valid experimental evidence has accumulated to clarify this problem it would seem more correct to acknowledge the existence of a pituitary hormone prolactin which *may* be luteotropic in the human female

The ovary responds to the gonadotropins with maturation of primordial follicles ovulation and corpus luteum formation The steroid hormones estrogen and progesterone which affect the growth and development of the endometrium and other accessory sex organs are elaborated by the ovary under the influence of the gonadotropins

Each primordial follicle consists of an ovum surrounded by a single layer of follicular cells known also as granulosa cells Maturation of the follicle is dependent upon the presence of the adenohypophyseal gonadotropins Proliferation of the granulosa cells and the formation of an antrum are the earliest morphologic changes observed in the growing follicle The interstitial elements of the ovary differentiate into the theca interna and the theca externa which encapsulate the follicle Prior to ovulation so-called preovulatory swelling occurs with the follicle increasing rapidly in size Ovulation then ensues with rupture of the ovarian capsule and extrusion of the ovum Following ovulation the ruptured follicle develops into a corpus luteum with transformation of the granulosa cells and theca interna cells into the lipid containing lutein cells The corpus luteum secretes both estrogen and progesterone and undergoes fibrotic degeneration if fertilization does not occur Prior to ovulation estrogen is the predominant hormone

The cyclic nature of adenohypophyseal and ovarian function is manifested in the endometrium by the proliferative secretory and bleeding phases of the menstrual cycle The characteristic histologic pattern of each of these endometrial phases is described elsewhere in this volume

As previously stated knowledge of the pituitary ovarian and endometrial cycle is essential to the understanding of various pathologic states associated with menstruation and reproduction Moreover an intelligent approach to the treatment of gynecologic endocrinopathies is dependent upon a thorough understanding of these hormonal relationships

Delayed Adolescent Development

The age at which the female secondary sex characteristics appear varies considerably These changes include maturation of the breasts evolution of typical feminine body contours and appearance of axillary and pubic hair The accessory sex characteristics usually have their inception between the eighth and the fourteenth year of life The age at which uterine bleeding begins also varies considerably The menarche is most often observed between the eleventh and the sixteenth year of life Because of these normal variations a precise definition of delayed adolescent

pituitary gland remains in doubt Harris[6] postulates that stimuli excite the hypothalamus to secrete a neurohumor of unknown composition This neurohumor is then transported by way of the hypophyseal venous portal system to excite or inhibit the secretory elements of the adenohypophysis

Finally primary amenorrhea as a clinical symptom may result from emotional trauma The psychogenic manifestations responsible for amenorrheic states vary from relatively superficial psychoneuroses to deep seated psychopathic states

The diagnostic evaluation and clinical management of patients with either primary or secondary amenorrhea are similar For this reason the essential diagnostic procedures and therapeutic regimes applicable to patients with primary amenorrhea are included in the following section of this chapter

Secondary Amenorrhea

The term secondary amenorrhea designates the cessation of uterine bleeding in patients who have previously menstruated The length of time which should elapse without external signs of uterine bleeding before consideration is given to secondary amenorrhea is difficult to define In general however if a patient has not menstruated for a period equivalent to three or four of her menstrual cycles investigation of the amenorrhea is warranted

Certain physiological states predispose to amenorrhea The possibility of pregnancy must always be entertained Menstruation is repressed for varying periods of time during the puerperium and during the lactational period

Periods of amenorrhea variable as to their duration are not uncommonly noted in the postmenarchal era and reflect normal fluctuations in ovarian activity The majority of these adolescent girls will resume perfectly normal menstrual periods in due course Patients in the premenopausal era not uncommonly manifest amenorrhea as a reflection of waning ovarian activity

Obstructive lesions involving the cervix may be responsible for states of secondary amenorrhea Cervical stenosis resulting in mechanical impediment to the drainage of uterine secretions may follow electrocauterization conization tracheloplasty or radiation chemical burns or infectious processes The cessation of menstruation may result from the destruction of endometrial tissue Irradiation with radioactive substances or x rays utilized in the treatment of malignancies of the genital tract produces secondary amenorrhea Destruction of the endometrium has also been reported following severe infections In most instances however the consequence of irradiation and infection is twofold In addition to the necrotizing effect on the endometrium destruction of the more distant ovarian tissue also ensues

Patients manifesting secondary amenorrhea on the basis of varying degrees of ovarian failure comprise by far the largest group encountered in clinical practice Hypo-ovarianism in most instances reflects either inadequate or improper stimulation by the adenohypophyseal gonadotropins or an inherent unresponsiveness of the ovarian tissue to normal gonadotropic stimulation The cause of ovarian failure in the presence of normal hypophyseal and hypothalamic function remains obscure Patients with relative ovarian failure often manifest dysfunctional uterine bleeding prior to the inception of the secondary amenorrheic state The underlying pathological process is often one of gradual ovarian failure The precise mechanisms involved in the pathogenesis of ovarian dysfunction remain to be elucidated Certain patients with hypogonadism demonstrate the Stein Leventhal syndrome which is discussed fully in another section of this chapter

A variety of interesting endocrinopathies result from hypofunction or hyperfunction of the adenohypophysis Despite the complex nature of the adenohypophyseal pattern of hormone secretion a remarkably good accounting may be made of the symptomatology and metabolic aberrations of these endocrinopathies in terms of the known actions of the tropic hormones

Extensive septic necrosis of the adenohypophysis following embolism leads to panhypopituitarism This syndrome is known variously as Simmonds disease or pituitary cachexia The so-called Sheehan s syndrome is an interesting variation of this disease and has its onset in the puerperium as a result of postpartum hemorrhage and obstetric shock

Amenorrhea may result from functioning pituitary tumors such as eosinophilic and basophilic adenomas or from nonfunctioning tumors such as chromophobe adenomas or craniopharyngiomas

Hyperadrenocorticism in man results from certain pathological processes involving the pituitary gland or the adrenal cortex (Cushing s disease and Cushing s syndrome respectively) A state of hypercorticism may also be induced in man by the administration of the adrenocorticotropic hormone (ACTH) or certain adrenal steroids In female patients with hyperadrenocorticism regardless of the etiology dysfunctional bleeding and amenorrhea comprise part of the clinical syndrome Inhibition of the adenohypophyseal release of the gonadotropic hormones by the elevated titer of adrenal steroids has been proposed as the mechanism responsible for the abnormal uterine bleeding and amenorrhea However recently reported data from animal experimentation suggests that the inhibition of gonadotropic activity which accompanies states of hyperadrenocorticism

toward the development of secondary sex characteristics and the initiation of cyclic bleeding

Primary Amenorrhea

The term amenorrhea denotes the absence or cessation of uterine bleeding and defines a clinical symptom rather than a clinical diagnosis The expression primary amenorrhea usually designates failure of the menarche to occur by the eighteenth year of life

Patients with primary amenorrhea may manifest normal secondary sex characteristics In most instances however primary amenorrhea is associated with so called hypogonadism that is underdevelopment of the mammae sparse distribution of pubic and axillary hair and retardation in the maturation of the genitalia

Etiological factors concerned with amenorrhea are more easily determined if attention is focused on the various anatomical sites important to the initiation of the menstrual process or to the transport of menstrual fluid These anatomical sites include the vaginal tract the uterus the ovaries the pituitary gland and the hypothalamus

Mechanical impediment to the drainage of uterine secretions may occur in the vaginal tract or in the cervical canal Stenosis in these areas results from congenital anomalies or from infectious processes An imperforate hymen is perhaps the most frequently encountered mechanical obstruction in patients manifesting primary amenorrhea In this condition the absence of uterine bleeding is associated with intermittent attacks of vaginal abdominal and lumbosacral pain A palpable pelvic mass is often discernible The condition if unattended leads progressively to hematocolpos and hematometra Diagnosis of an imperforate hymen is not difficult and treatment consists of the establishment of adequate drainage by hymenotomy Patients with obstructive lesions of the vagina or cervix usually manifest normal ovarian and endometrial function However continued normality depends upon correction of the mechanical impediment

Agenesis of the vagina resulting from aplasia of the vaginal segments of the Mullerian ducts is occasionally encountered as the cause of primary amenorrhea In most instances the uterus is also congenitally missing but exceptions to this have been reported Patients with congenital absence of the vagina usually possess normal secondary sex characteristics indicating ovarian function and estrogenic stimulation Diagnosis of this condition is not difficult Treatment consists of surgical correction of the anomalous condition In most instances it is deemed advisable to postpone definitive surgery until the patient contemplates marriage The prognosis for satisfactory marital relationships is excellent providing careful attention is paid to surgical technic and postoperative care

Congenital absence of the uterus though not common is occasionally encountered by the practicing physician or gynecologist This anomaly results from abnormal development of those elements of the Mullerian canals destined to form the uterus The vagina in these patients may also be absent or may be essentially normal

Primary amenorrhea results from absolute ovarian failure due to ovaries congenitally absent or rudimentary Developmental abnormalities of the ovaries are discussed in another section of this chapter

Congenital anomalies of the urinary tract are often associated with developmental abnormalities of the genital system For this reason intravenous pyelography should always be included in the study of such cases

The majority of patients manifesting primary amenorrhea as a clinical symptom possess adequate ovarian tissue but for one reason or another lack adequate estrogenic stimulation Absent or diminished ovarian secretory activity results in most cases from an inherent ovarian defect or from abnormalities of the hypophysis or the hypothalamus The underlying cause of ovarian unresponsiveness in the presence of normal gonadotropic stimulation remains obscure

The complex metabolic derangement resulting from panhypopituitarism is a consequence of deficiencies of all of the tropic hormones of the adenohypophysis Panhypopituitarism in infants or acquired at an early age is characterized by defects in growth and development The ovaries remain small uterine bleeding is lacking and the secondary sex characteristics fail to develop due to the lack of gonadotropic stimulation Panhypopituitarism may be congenital or the condition may result from cysts tumors infection infarction or malnutrition

In very rare instances adenohypophyseal function is apparently normal except for a specific deficiency of the gonadotropic hormones The pathogenesis of this condition remains to be elucidated

Gonadal dysfunction in man is not infrequently associated with hypothalamic lesions such as tumors or cysts However even in the absence of demonstrable pathology amenorrhea may result from abnormal hypothalamic function Impressive evidence has been accumulated to suggest that the higher brain centers influence sexual patterns However the precise mechanisms of this neural control remain obscure The influence of the hypothalamus over adenohypophyseal function may be exerted by direct nervous connections or through a neurohumoral pathway involving the hypothalamico hypophyseal venous portal system The existence of direct nervous connections between the hypothalamus and the

drawal flow if it is to occur will be noted 2 8 days after the final injection of the hormone

Failure of withdrawal bleeding following progesterone administration may also be due to pregnancy Careful examination of the internal genitalia and the employment of pregnancy tests are helpful in establishing the diagnosis of an early gestation

Reference is frequently made in the medical literature to persistence of the corpus luteum in a functional state as a distinct clinical entity Existing evidence suggests that in man the corpus luteum possesses an inherent life span and considerable doubt can be cast on the persistence of luteal function beyond this period However if one acknowledges the existence of persistent corpora lutea inhibition of the adenohypophysis due to continued ovarian steroid elaboration can result in a state of amenorrhea Quite obviously withdrawal bleeding would not follow progesterone administration Urinary pregnanediol assays would be helpful in differentiating this condition from absolute ovarian failure

The concentration of estrogenic substances in the urine and the serum reflects the degree of ovarian activity Determination of the estrogenic titer in body fluids would be invaluable in the diagnosis of certain of the endocrinopathies affecting the female reproductive system However methods presently existing for the quantification of estrogenic steroids in body fluids are fraught with technical difficulties and the results obtained are often grossly inaccurate For this reason the employment of these bioassay technics is not practical at the present time

Women manifesting ovarian failure excrete adenohypophyseal gonadotropins at a greater than normal rate and without cyclic fluctuations The gonadotropin present in the urine of such patients is predominantly follicle stimulating in nature Methods for the bioassay of FSH in urine are utilized in the evaluation of amenorrheic patients Thus the diagnosis of absolute ovarian failure can be established in a patient who (a) does not respond to progesterone administration with withdrawal bleeding and (b) demonstrates an elevated FSH titer in the urine

Various abnormalities of the pituitary gland as stated may be responsible for amenorrheic states Secretion of the adenohypophyseal gonadotropins is absent or significantly diminished in patients with abnormalities of the pituitary gland For this reason the ovaries and hence the endometrium receive no hormonal stimulation and amenorrhea ensues With lesions of the pituitary gland (a) the concentration of FSH in the urine is low or absent (b) withdrawal flow does not follow the administration of progesterone but (c) the endometrium does respond to estrogenic stimulation with withdrawal bleeding

Roentgenographic evaluation of the sella turcica is imperative in the clinical evaluation of all amenorrheic states Neoplasms of the pituitary gland and adjacent structures manifest characteristic x ray findings Neurological and ophthalmological consultation is essential if pituitary or hypothalamic tumors are suspected

Lesions of the hypothalamus are characterized by the failure of estrogenic elaboration in the presence of normal levels of FSH The secretion of estrogenic substances depends upon adequate ovarian stimulation by two of the gonadotropic hormones FSH and LH Convincing and substantial evidence has been accumulated to indicate that the adenohypophyseal release of LH is under the control of the hypothalamus In conditions of hypothalamic dysfunction (a) the endometrium is intact as evidenced by withdrawal flow following estrogen treatment (b) the ovaries are not active as shown by the absence of uterine bleeding following the administration of progesterone and (c) the concentration of FSH in the urine is normal Determination of the titer of LH in body fluids would be most helpful in the diagnosis of hypothalamic lesions unfortunately satisfactory methods for the bioassay of LH are not presently available

Two fundamental concepts both equally important should be stressed in the clinical management of patients with primary or secondary amenorrhea First the absence of menstruation or the cessation of previously established menstruation does not in the majority of patients represent a serious threat to their well being Secondly in the absence of proved organic lesions amenorrhea as a clinical symptom warrants treatment only as it relates to disturbed emotional states or to infertility Many physicians seem compelled to initiate cyclic uterine bleeding before adequately investigating the patient to determine the existence of serious organic lesions

The procedures available to the physician for the clinical evaluation of patients with amenorrhea have been outlined In addition to investigating the functional integrity of the endometrium the ovaries the pituitary glands and the hypothalamus attention should also be directed toward the uncovering of systemic diseases abnormalities of other endocrine organs and psychogenic factors Appropriate treatment when indicated should be initiated if such pathologic states exist In the absence of proved organic lesions cyclic endocrine therapy may be indicated if the failure of uterine bleeding to occur emotionally disturbs the patient Under these circumstances estrogenic preparations are administered according to the dosage schedules outlined in the section devoted to delayed adolescent development and delayed menarche Cyclic uterine bleeding resulting from withdrawal flow is easily accomplished in these patients providing inherent endometrial unresponsiveness does not exist

occurs at the ovarian level rather than at the adeno hypophyseal level[4]

Amenorrhea is also a manifestation of Addison's disease or adrenal hypocorticism

In certain patients ovarian failure and amenorrhea result from a specific deficiency of the adenohypophyseal gonadotropins The etiological factors responsible for this condition remain obscure Inhibition of the adenohypophyseal release of the gonadotropins resulting in the cessation of uterine bleeding follows the administration of large doses of estrogen progesterone or testosterone The increased endogenous secretion of ovarian steroids resulting from certain functioning tumors of the ovaries inhibit gonadotropic activity in a similar fashion Neoplasms of this nature are discussed elsewhere in this chapter

Malnutrition due to starvation or chronic wasting disease may result in hypofunction of the pituitary gland Secondary amenorrhea following rigorous and severe reducing diets is not infrequently encountered and probably represents a state of relative hypopituitarism The cessation of uterine bleeding is a clinical feature of anorexia nervosa

Lesions of the hypothalamus may also be responsible for alterations in gonadotropic activity The functional relationship of the hypothalamus and the adenohypophysis has previously been discussed in this chapter

Abnormal psychogenic states either cause or contribute to the development of secondary amenorrhea and other abnormalities of menstrual and reproductive function The cessation of menstruation not uncommonly results from the fear of pregnancy incompatible marital relationships psychosexual conflicts financial worry intolerable family situations and other traumatic psychiatric states Although the precise pathogenesis remains obscure the *modus operandi* for the development of abnormal reproductive function in psychogenic states may be associated with hypothalamic dysfunction For this reason the so called emotional amenorrhea has also been termed hypothalamic amenorrhea

The absence of menstruation or the cessation of previously established menstruation does not in the vast majority of patients represent a serious threat to their well being or longevity Amenorrhea as a clinical symptom warrants evaluation in all patients A concerted effort should be made to disclose underlying systemic disease to uncover lesions of the various endocrine target organs and to investigate abnormalities of the higher brain *In the absence of proved organic lesions amenorrhea as a clinical symptom warrants treatment only as it relates to infertility or to disturbed emotional states which not infrequently arise from the absence or the cessation of uterine bleeding*

The evaluation of patients with either primary or secondary amenorrhea necessitates thorough testing of the functional integrity of each link in the chain of menstrual events that is the endometrium the ovaries the pituitary gland and the hypothalamus Investigation of other endocrine systems such as the thyroid and adrenal glands and a search for underlying systemic disease may also be necessary

It would seem unnecessary to emphasize the imperativeness of a complete history and physical examination yet the number of patients in which these simple procedures have been totally ignored by the practicing physician is disturbing and appalling

The authors routinely obtain vaginal smears from all gynecologic patients *This is not an essential procedure* in the evaluation of amenorrheic states and depends upon the availability of trained personnel for scrutiny of the smears The cytological characteristics of the desquamated cells reflect not only epithelial atypism but also the degree and character of the endocrine stimulation of the genital tract

Endometrial biopsies obtained simply and easily in the physician's office are very valuable in the study of patients manifesting amenorrhea Needless to say the possibility of an intra uterine pregnancy should be entertained prior to undertaking this procedure

The investigation of an intrinsic endometrial defect *as the cause of amenorrhea is not difficult* Endometrium atrophied as a result of deficient ovarian secretion maintains an inherent faculty to respond to estrogenic stimulation regardless of the duration of the atrophy Withdrawal uterine bleeding following the administration of adequate amounts of estrogen is a well established endocrine phenomenon If uterine bleeding is not manifested after adequate estrogenic therapy the diagnosis of an intrinsic endometrial defect can be established For this test diethylstilbestrol 5 mgm daily by mouth for 24 days or ethinyl estradiol 0 05 mgm twice daily by mouth for 24 days is employed Withdrawal flow if it is to occur will be noted within 10 12 days after cessation of the estrogenic treatments

The utilization of known endocrinologic relationships is helpful in differentiating between absolute and relative ovarian failure as the basis for amenorrhea Withdrawal uterine bleeding follows the administration of adequate amounts of progesterone providing the endometrium is stimulated or primed with estrogen If uterine bleeding follows the administration of progesterone estrogen to some degree is being elaborated by the ovary If withdrawal flow does not follow the administration of progesterone complete ovarian failure must be considered For this test progesterone 50 or 100 mgm is administered intramuscularly on two successive days With

drawal flow if it is to occur will be noted 2 8 days after the final injection of the hormone

Failure of withdrawal bleeding following progesterone administration may also be due to pregnancy Careful examination of the internal genitalia and the employment of pregnancy tests are helpful in establishing the diagnosis of an early gestation

Reference is frequently made in the medical literature to persistence of the corpus luteum in a functional state as a distinct clinical entity Existing evidence suggests that in man the corpus luteum possesses an inherent life span and considerable doubt can be cast on the persistence of luteal function beyond this period However if one acknowledges the existence of persistent corpora lutea inhibition of the adenohypophysis due to continued ovarian steroid elaboration can result in a state of amenorrhea Quite obviously withdrawal bleeding would not follow progesterone administration Urinary pregnanediol assays would be helpful in differentiating this condition from absolute ovarian failure

The concentration of estrogenic substances in the urine and the serum reflects the degree of ovarian activity Determination of the estrogenic titer in body fluids would be invaluable in the diagnosis of certain of the endocrinopathies affecting the female reproductive system However methods presently existing for the quantification of estrogenic steroids in body fluids are fraught with technical difficulties and the results obtained are often grossly inaccurate For this reason the employment of these bioassay techniques is not practical at the present time

Women manifesting ovarian failure excrete adenohypophyseal gonadotropins at a greater than normal rate and without cyclic fluctuations The gonadotropin present in the urine of such patients is predominantly follicle stimulating in nature Methods for the bioassay of FSH in urine are utilized in the evaluation of amenorrheic patients Thus the diagnosis of absolute ovarian failure can be established in a patient who (a) does not respond to progesterone administration with withdrawal bleeding and (b) demonstrates an elevated FSH titer in the urine

Various abnormalities of the pituitary gland as stated may be responsible for amenorrheic states Secretion of the adenohypophyseal gonadotropins is absent or significantly diminished in patients with abnormalities of the pituitary gland For this reason the ovaries and hence the endometrium receive no hormonal stimulation and amenorrhea ensues With lesions of the pituitary gland (a) the concentration of FSH in the urine is low or absent (b) withdrawal flow does not follow the administration of progesterone but (c) the endometrium does respond to estrogenic stimulation with withdrawal bleeding

Roentgenographic evaluation of the sella turcica is imperative in the clinical evaluation of all amenorrheic states Neoplasms of the pituitary gland and adjacent structures manifest characteristic x ray findings Neurological and ophthalmological consultation is essential if pituitary or hypothalamic tumors are suspected

Lesions of the hypothalamus are characterized by the failure of estrogenic elaboration in the presence of normal levels of FSH The secretion of estrogenic substances depends upon adequate ovarian stimulation by two of the gonadotropic hormones FSH and LH Convincing and substantial evidence has been accumulated to indicate that the adenohypophyseal release of LH is under the control of the hypothalamus In conditions of hypothalamic dysfunction (a) the endometrium is intact as evidenced by withdrawal flow following estrogen treatment (b) the ovaries are not active as shown by the absence of uterine bleeding following the administration of progesterone and (c) the concentration of FSH in the urine is normal Determination of the titer of LH in body fluids would be most helpful in the diagnosis of hypothalamic lesions unfortunately satisfactory methods for the bioassay of LH are not presently available

Two fundamental concepts both equally important should be stressed in the clinical management of patients with primary or secondary amenorrhea First the absence of menstruation or the cessation of previously established menstruation does not in the majority of patients represent a serious threat to their well being Secondly in the absence of proved organic lesions amenorrhea as a clinical symptom warrants treatment only as it relates to disturbed emotional states or to infertility Many physicians seem compelled to initiate cyclic uterine bleeding before adequately investigating the patient to determine the existence of serious organic lesions

The procedures available to the physician for the clinical evaluation of patients with amenorrhea have been outlined In addition to investigating the functional integrity of the endometrium the ovaries the pituitary glands and the hypothalamus attention should also be directed toward the uncovering of systemic diseases abnormalities of other endocrine organs and psychogenic factors Appropriate treatment when indicated should be initiated if such pathologic states exist In the absence of proved organic lesions cyclic endocrine therapy may be indicated if the failure of uterine bleeding to occur emotionally disturbs the patient Under these circumstances estrogenic preparations are administered according to the dosage schedules outlined in the section devoted to delayed adolescent development and delayed menarche Cyclic uterine bleeding resulting from withdrawal flow is easily accomplished in these patients providing inherent endometrial unresponsiveness does not exist

The therapeutic management of sterility patients manifesting amenorrhea deserves special note Relative ovarian failure is the most frequent cause of the amenorrhea and hence the infertility in the vast majority of these patients There is no specific treatment for relative ovarian failure and the sterility which ensues Nonetheless therapeutic trials with estrogen and progesterone seem warranted Unfortunately treatment with these endocrine preparations almost always fails to initiate ovulation or to correct the infertility

Two therapeutic regimens have been tried by various investigators Estrogenic preparations have been employed for the purpose of stimulating the hypothalamico hypophyesal system to secrete gonadotropic substances in concentrations capable of arousing the ovaries to active function For this purpose diethylstilbestrol 0 1 mgm daily by mouth or ethinyl estradiol 0 02 mgm daily by mouth is administered Desiccated thyroid 65 mgm daily by mouth is usually given in conjunction with the estrogenic substances The clinical results from this treatment program have been disappointing

Endocrine preparations are also employed to remove gonadotropic stimulation of the ovaries The *rationale of this treatment utilizes the observation* that estrogenic substances administered in large doses block the adenohypophyseal release of the gonadotropins Existing evidence suggests that the gonadotropic hormones are accumulated or stored in higher concentrations in the pituitary gland during this period of endocrine therapy Cessation of the treatment theoretically at least releases the estrogenic blockage and results in a sudden burst of gonadotropic activity The desired effect of this so called rebound phenomenon is to stimulate the ovaries sufficiently to promote ovulation and normal menstrual function Unfortunately the results are disappointingly poor The authors have employed cyclic estrogen and progesterone treatment with such patients Diethylstilbestrol up to 25 mgm daily by mouth or ethinyl estradiol 0 05 mgm three times daily by mouth is administered for 24 consecutive days Progesterone 50 mgm or 100 mgm is injected intramuscularly on the twenty third and twenty *fourth days of the treatment Therapy is then* stopped and the patient experiences withdrawal uterine bleeding two to six days later Administration of the estrogenic preparation is commenced on the fourth day of flow and continues through the twenty fourth day of the artificial cycle Progesterone is again injected on the twenty third and twenty fourth days This therapeutic program is continued for three artificial cycles At the conclusion of the final withdrawal flow basal temperature readings are recorded by the patient and coitus is advised if an ovulatory type of temperature curve is noted The administration of progesterone in the dosage schedule employed does not contribute appreciably to the adenohypophyseal blockade However withdrawal uterine bleeding more nearly approximating normal menstruation ensues from the combined use of *estrogen and progesterone Uterine* bleeding which follows the cessation of estrogenic therapy alone is often profuse and for this reason disturbing to the patient The administration of progesterone eliminates this profuse bleeding

DYSFUNCTIONAL UTERINE BLEEDING

Introduction

The term dysfunctional uterine bleeding was first employed by William P Graves [5] to describe conditions of abnormal endometrial bleeding in which organic lesions could not be demonstrated *The expression functional uterine bleeding* is used by many physicians to define these states of abnormal flowing However the term functional in the usual sense implies normal and proper fulfillment of duty Designating abnormal endometrial bleeding as functional uterine bleeding seems therefore inconsistent For *this reason the authors prefer the expression dysfunctional uterine bleeding*

The incidence of dysfunctional uterine bleeding is very high and abnormal uterine flowing may occur at any age between the menarche and the menopause The highest incidence is noted in the post *menarchal and premenopausal periods that is* during the initiation and the decline of ovarian function

The underlying abnormality responsible for dysfunctional uterine bleeding may originate in the higher brain centers the ovaries or the endometrium Normal menstrual cycles are characterized by proper balances between the protein adenohypophyseal gonadotropins and the steroid ovarian hormones Dysfunctional uterine bleeding probably results from an imbalance of these endocrines rather than a deficiency of pituitary and ovarian hormones *per se*

The ovary may be implicated as the etiological factor in most cases of abnormal uterine bleeding *Abnormal ovarian activity may* result from inherent ovarian dysfunction or secondarily from functional derangements of the hypothalamus or hypophysis

Abnormal uterine bleeding is almost always associated with endometrial growth patterns in which the effect of progesterone is completely lacking or markedly deficient that is most dysfunctional bleeding occurs from proliferative endometrium When excessive uterine bleeding is associated with normal secretory endometrium the etiology is usually organic in nature

A careful history particular attention being paid

for an accurate description of the bleeding is essential and helpful in the investigation of dysfunctional uterine bleeding General physical examination including careful evaluation of the pelvic structures is imperative Appraisal of the internal genitalia is accomplished in adolescent girls and young unmarried women by means of careful rectal examination A search for the presence of any anemia should be undertaken

One important point in the management of patients with dysfunctional flow should be stressed Endocrine therapy in order to be effective must be based on accurate diagnosis of the underlying hormonal defect There is no place for trial or empirical therapy in the treatment of abnormal uterine bleeding states In practically all instances an accurate diagnosis is possible providing endometrial tissue is obtained at the proper time for histologic study Endometrial tissue adequate for study may be recovered by utilizing the simple office procedure of endometrial biopsy or the operative technic of dilatation and curettage In order to insure an accurate histologic diagnosis endometrium should be obtained on the first day of bleeding or during the period of abnormal flowing depending upon the bleeding pattern An endometrial biopsy because it is an office procedure provides a much simpler method for obtaining tissue at the proper time Dilatation and curettage necessitate more elaborate preparations and are often difficult to schedule properly especially in patients with grossly irregular periods However curettage has the advantage of being therapeutic in some of the cases of dysfunctional uterine flow Advocates of curettage for patients with abnormal bleeding argue that the pathologist is provided with considerably more tissue for study than can be recovered from endometrial biopsies However it should be emphasized that very adequate and representative samples of endometrial tissue can be obtained from endometrial biopsies providing care is taken during the procedure The most important criterion in the diagnosis and the management of dysfunctional flow is proper timing not only of the diagnostic procedures but also of the therapeutic regimes which are initiated

In addition to curettage for the treatment of abnormal bleeding the physician possesses in his armamentarium the steroid hormones estrogen and progesterone Treatment with these hormones often restores the continuity and balance of the pituitary ovarian endometrial axis resulting in normal metabolic processes and the regulation of bleeding The gonadotropic hormones either pituitary or placental *in origin have been universally unsatisfactory in the* treatment of dysfunctional bleeding problems

Attention in the diagnosis and management of abnormal bleeding states should also be paid to systemic diseases and to constitutional abnormalities such as obesity and malnutrition Endocrinopathies involving other target organs may also affect normal menstrual patterns Psychogenic and emotional trauma may predispose to dysfunctional flow and evaluation of the patient s psychiatric status is often indicated

Uterine Bleeding Associated with Ovulation

The process of ovulation occurs in most women *14* days *plus or minus* 2 days prior to the onset of the next menstrual period Convincing evidence has accumulated which suggests that the progravid phase of the menstrual cycle is relatively constant in length 14 ± 2 days whereas the proliferative phase varies considerably in length Uterine bleeding not infrequently occurs during the ovulation period especially in women in the latter half of their reproductive lives So called ovulatory bleeding may be very slight or moderately profuse The staining *usually persists for only one to three days occasion*ally however the bleeding continues for longer periods and may linger until the onset of the next menstrual period Endometrial biopsies obtained during the bleeding phase disclose late proliferative or early progravid endometrium

This type of uterine bleeding probably results from a breakdown of the endometrium at the time of ovulation Rupture of the ovarian follicle with extrusion of the ovum into the peritoneal cavity interferes with the vascular integrity of the follicle and developing corpus luteum a temporary decrease in the circulating titer of ovarian hormones results Ovulatory bleeding is an example of withdrawal uterine bleeding

The diagnosis of this type of bleeding is estab*lished from a careful history Treatment consists of* reassurance of the patient providing that other abnormalities are not discernible and that this type of abnormal flow represents the only bleeding irregularity the patient manifests

Premenstrual Staining

Premenstrual staining is frequently associated with endometrial polyps and submucous leiomyomata However endocrine imbalances also predispose to premenstrual staining Normally menstruation follows a sharp decline in the elaboration of steroids from the corpus luteum Premenstrual staining results from premature breakdown or teetering of the *endometrium due to a gradual decline or fluctuation* in the level of the ovarian hormones The underlying pathology is usually early failure of the corpus luteum Histologically the endometrium from such

patients often manifests some degree of progestational immaturity

Definitive treatment of this condition is often unnecessary However if premenstrual staining is disconcerting to the patient intravaginal progesterone may be administered with beneficial effects *Progesterone treatment is commenced eight days before the* onset of the next menstrual period is expected and the therapy is continued for four consecutive days Suppositories containing 50 mgm of progesterone are inserted intravaginally daily for the duration of the treatment Therapy is directed toward the support of the failing endometrium Should uterine staining occur while the patient is receiving progesterone underlying organic pathology is usually present and curettage is indicated

Normal Uterine Bleeding Occurring at Shorter Than Usual Intervals

Although the length of the average menstrual cycle is 28 days cycles occurring at regular 21 36 day intervals should not be considered abnormal unless they represent a deviation from the established pattern of the patient or unless the patient presents herself as a sterility problem As previously stated the duration of the progravid phase of the menstrual cycle is relatively constant whereas the length of the proliferative phase is somewhat more variable

Uterine bleeding at relatively frequent intervals is not uncommonly noted in the postmenarchal period In these patients shortening of the cycles results from fluctuations in ovarian activity definitive therapy is usually unnecessary Normal uterine bleeding occurring at shorter than usual intervals is not infrequently noted in the premenopausal era and again represents fluctuations in ovarian activity Definitive therapy is unnecessary in the majority of these patients

If uterine bleeding at shorter than usual intervals represents a deviation from the established normal pattern of the patient or if the patient presents herself as an infertility problem definitive therapy is warranted Under these circumstances endometrial biopsies obtained at the onset of uterine bleeding are helpful in determining the functional status of the ovaries and the endometrium Contrary to popular belief curettage will not rectify the shortened cycles *The most satisfactory results in the treatment* of these patients come from the use of so called artificial cycles Diethylstilbestrol up to 25 mgm daily by mouth or ethinyl estradiol 0 05 mgm by mouth three times daily is commenced on the fourth day of the uterine flow and is continued for 21 days that is through the twenty fourth day of the artificial cycle Progesterone 50 mgm intramuscularly or intravaginally is administered on days 23 and 24 of the cycle Withdrawal uterine bleeding usually commences in two to six days *after cessation of the* therapy Treatment is started again on the fourth day as outlined and is continued for three such artificial cycles Normal menstrual cycles may be established following this course of therapy If the patient is an infertility problem basal temperature determinations are recorded and a planned conception program is initiated

Administering estrogens in these pharmacological doses blocks the adenohypophyseal release of gonadotropins and secondarily results in temporary cessation of ovarian function The release of the pituitary gonadotropins following this type of therapeutic approach may result in normally balanced pituitary *ovarian endometrial relationships A so-called re*bound phenomenon with an actual increase in the secretion of the pituitary gonadotropins may result although definitive evidence to support this concept has not been established Such therapy may also render the ovaries more responsive to gonadotropic stimulation

It should be emphasized that the use of artificial cycles does not represent a panacea for all menstrual irregularities *but under certain circumstances* such therapy warrants a trial especially with infertility patients in which no definite cause for the sterility can be uncovered Finally progesterone is employed only to insure these patients more normal bleeding during the period of withdrawal flow Progesterone in the dosage schedule outlined does not contribute to the adenohypophyseal blockade

Normal Uterine Bleeding Occurring at Longer Than Usual Intervals

As stated the length of the menstrual cycle is variable A precise definition of prolonged cycles is diffi*cult to establish Normal uterine bleeding* occurring at longer than usual intervals is not infrequently encountered in the postmenarchal and the premenopausal periods and represents fluctuations in ovarian activity However prolongation of the length of the menstrual cycle warrants investigation in *sterility* patients and in patients in whom this pattern represents a change from previously established menstrual patterns Lengthening of the menstrual cycles may herald the approach of secondary amenorrhea as a clinical symptom Etiological factors to be considered in patients manifesting prolonged cycles are similar to those discussed in the section of this chapter devoted to secondary amenorrhea Although *the precise pathogenesis* remains obscure relative ovarian failure is probably the causative factor in the vast majority of these patients Systemic disease malnutrition obesity dysfunction of other endocrine

organs and psychic disturbances should also be considered

The diagnostic and therapeutic procedures applicable to patients with prolonged cycles are similar to those discussed for the evaluation and management of secondary amenorrhea

Profuse or Prolonged Uterine Bleeding at Normal Intervals

The magnitude and the duration of uterine bleeding vary considerably among patients and a concise definition of profuse or prolonged flowing is difficult to establish Moreover what seems to be excessive bleeding to one patient is considered perfectly normal flowing by another Most women require a change of protective pads three or four times a day during the height of menstruation If more frequent changing is found necessary by the patient the uterine bleeding is usually considered profuse The diagnosis of prolonged uterine bleeding should be entertained if the flow persists for more than eight to ten days The presence or the absence of an anemic state is helpful in determining the profuseness of uterine bleeding Finally if the patient is disturbed with the amount and duration of flowing during each bleeding phase diagnostic and therapeutic steps should be initiated

The initial evaluation of patients with profuse and prolonged uterine bleeding should be directed toward the uncovering of organic lesions Retained products of conception polyps carcinoma leiomyomata pelvic inflammatory disease blood dyscrasias systemic diseases constitutional defects and psychogenic factors must all be included in the differential diagnosis Profuse and prolonged uterine bleeding in patients without demonstrable organic or psychogenic pathology will be considered here

Histologic examination of the endometrium obtained from patients with profuse or prolonged uterine bleeding at normal intervals usually discloses some degree of progesterone activity Frequently however examination of the endometrium will disclose rather marked progestational immaturity suggesting deficient progesterone secretion from the corpus luteum Profuse and prolonged uterine bleeding results from an imbalance of the normal pituitary-ovarian relationships However the precise endocrine abnormalities responsible for such uterine bleeding remain obscure Our knowledge concerning the fundamental hormonal interactions of the normal menstrual cycle is meager Careful evaluation of the existing evidence clearly indicates that our present concepts of the endocrine relationships of the human menstrual cycle are based largely on animal experimentation grossly inaccurate methods of bioassay and sweeping supposition The fundamental etiological mechanisms responsible for dysfunctional uterine bleeding will continue to remain obscure until such time as accurate methods for the isolation and quantification of the pituitary and ovarian hormones in body fluids are devised

The clinical management of patients with profuse and prolonged uterine flowing is directed toward the disclosure of existing organic pathology and the cessation of bleeding Satisfactory treatment is dependent upon an accurate diagnosis of the type of endometrium from which the patient is bleeding Endometrium for histologic study may be obtained by either endometrial biopsy or curettage Adolescent girls in the postmenarchal period and young unmarried women with profuse and prolonged bleeding at regular intervals represent a notable exception to the dictum that therapy should not be initiated without first obtaining endometrial tissue for study In these age groups it is often deemed inadvisable and unnecessary to subject the patient to endometrial biopsy or curettage Satisfactory clinical management is usually effected with hormonal therapy alone However if the response to treatment is not prompt curettage should be performed to rule out local causes of abnormal uterine bleeding

The diagnostic and therapeutic armamentarium of the physician includes endometrial biopsy curettage and hormonal therapy The endometrial biopsy is never therapeutic in patients with dysfunctional uterine bleeding but does provide a convenient and satisfactory method for obtaining endometrial tissue for histologic study The criticism is often raised that adequate and representative endometrium cannot be obtained with this method The authors express the opinion that with care adequate and representative samples of endometrial tissue can be recovered with a minimum of discomfort to the patient The endometrial biopsy technic is not proposed as a substitute for examination under anesthesia and dilatation and curettage However temporal relationships are important in the establishment of an accurate diagnosis in patients with dysfunctional bleeding problems If endometrium is recovered during the nonbleeding phase the probability of an accurate diagnosis is greatly diminished To be sure the diagnosis of endometrial carcinoma can be made during any phase of the cycle If establishing the diagnosis of cancer in a given patient is foremost in the mind of the physician the temporal relationships alluded to are obviously unimportant and should be completely disregarded However if dysfunctional uterine bleeding is suspected attention must be paid to recovery of the endometrium at the proper time The authors obtain endometrial tissue at the very onset of uterine bleeding or during the bleeding phase and utilize the endometrial biopsy technic for this purpose Dilatation and curettage if indicated can then be

scheduled at a time more convenient for both patient and physician. Which patients require dilatation and curettage for therapy is based on the individualization of each case; hard and fast rules cannot be established. However, the dictum that all patients with dysfunctional uterine bleeding must be curetted is not tenable.

The cessation of profuse or prolonged uterine bleeding occurring at regular intervals can often be effected with properly administered progesterone therapy. As previously stated, the vast majority of such patients usually bleed from endometrium manifesting some degree of progestational activity. The administration of progesterone supplements endogenously secreted progesterone and improves the integrity of the poorly secretory endometrium. Withdrawal of the hormone results in more rapid and more complete desquamation of the endometrium and hence more rapid hemostasis.

Certain practicalities in the management of patients with profuse and prolonged periods occurring at regular intervals warrant consideration. If the patient is first seen during the nonbleeding phase of the cycle, arrangements are made to secure an endometrial biopsy within eight to twelve hours after the onset of uterine bleeding. The physician may elect to observe the patient during this particular bleeding phase in order to evaluate the character and intensity of the flow, or he may decide to initiate therapy on the first day in an attempt to control the abnormal bleeding. If the former course is chosen, progesterone, 50 or 100 mgm. intramuscularly, is administered on the twenty-fourth day of the cycle. Uterine bleeding, usually normal in character, commences two to six days after the injection of progesterone. This therapeutic regime is continued for three cycles, the first day of flowing being considered the first day of the cycle. If intermenstrual bleeding or abnormal flowing occurs while the patient is receiving progesterone therapy, or if profuse and prolonged periods follow cessation of the treatment, some pathologic condition exists within the uterus and curettage is imperative.

If the physician decides to initiate therapy on the first day of bleeding in an attempt to control the abnormal flowing, an endometrial biopsy is obtained at the onset of the bleeding and concomitantly 100 mgm. of progesterone are administered intramuscularly. The patient should be cautioned of rather profuse flow in two to six days. Withdrawal of the progesterone is usually accompanied by more complete sloughing of the endometrium, rapid hemostasis and cessation of the abnormal bleeding. Intramuscular progesterone is then administered on the twenty-fourth day of the cycle as outlined above. This therapeutic program is continued for three menstrual cycles. Satisfactory clinical results usually ensue; if the profuseness persists or if intermenstrual staining is noted, curettage is mandatory.

If the patient is first seen during the actual phase of profuse or prolonged uterine bleeding, an endometrial biopsy is obtained for histologic study and 100 mgm. of progesterone administered intramuscularly. The excessive bleeding usually diminishes following the progesterone treatment, but the patient should be cautioned of more profuse withdrawal flow in two to six days after the endocrine therapy. Progesterone is then administered on the twenty-fourth day of three successive cycles as outlined above.

Patients with profuse and prolonged uterine bleeding at regular intervals may also be treated with estrogenic substances. Diethylstilbestrol, up to 25 mgm. daily by mouth, or ethinyl estradiol, 0.05 mgm. three times each day by mouth, is administered from the fourth through the twenty-fourth day of the cycle. This therapeutic program is continued for three such cycles. The rationale for the treatment of prolonged and profuse uterine bleeding with estrogens is twofold. Estrogen administered in these dosages effectively inhibits the adenohypophyseal release of the gonadotropins during the course of the therapy. When treatment with estrogens is halted, a more normal spectrum of gonadotropic elaboration often ensues. In addition, the administration of estrogenic preparations also increases the anabolic reaction of the endometrium. The clinical results with estrogenic therapy are not always satisfactory. If uterine staining occurs while the patient is undergoing treatment, curettage is indicated.

Combined estrogen-progesterone therapy may also be employed for the management of profuse or prolonged uterine bleeding occurring at regular intervals. Diethylstilbestrol, up to 25 mgm. daily by mouth, or ethinyl estradiol, 0.05 mgm. orally three times daily, is administered from the fourth through the twenty-fourth day of the cycle. Progesterone, 50 or 100 mgm., is given intramuscularly on the twenty-fourth day. Withdrawal flow usually commences in two to six days after cessation of the treatment. Estrogenic substances are commenced again on the fourth day of the bleeding and continued through the twenty-fourth day of the cycle. Progesterone is administered again on the twenty-fourth day. This therapeutic program is extended for three such cycles.

The development of newer progestins which are orally effective has simplified the treatment of some forms of amenorrhea and dysfunctional uterine bleeding. Although orally ingested progesterone is not completely deactivated, it requires a dose 20 times that of the parenteral form and 3 to 5 times that of the buccal or sublingual form. Ethisterone (anhydrohydroxyprogesterone) was described as an orally effec-

tive progestogen in 1938 but its dosage is similar to buccal or sublingual progesterone

In 1957 two compounds were introduced which were orally effective well tolerated and which in relatively small dosage could produce the biologic effects of parenterally administered progesterone They are 17 alpha-ethinyl 19 nortestosterone (norethindrone) * and 17 alpha-ethinyl 17 hydroxy 5(10) estren-3-one (norethnodrel) † The structural formulas for these preparations are shown in Figures 1 and 2 Both progestins are examples of the increased biological activity of steroids obtained when the methyl group at carbon 10 is removed (as in norethnodrel) or is replaced by hydrogen (as in norethindrone) Because of the removal of the 19 carbon these drugs are now termed the 19 Nor steroids Both have been found to possess the thermogenic effects of progesterone as well as the ability to produce secretory changes in the endometrium and vaginal cells as well as inhibiting the fern leaf pattern of the cervical mucosa

Dosage Amenorrhea If adequate estrogen priming is present cyclic bleeding from a secretory endometrium may be obtained by the administration of 10-20 mg of norethindrone or norethnodrel for 20 consecutive days Within 2-4 days after stopping the medication a bleeding episode lasting from 4 to 5 days will occur If adequate estrogen priming has not been present bleeding will not occur and in such cases the preliminary use of an estrogen is usually necessary for an effective response It is suggested that artificial cycles of this type be carried out for 3-4 months Not infrequently for reasons unknown, spontaneous menstruation occurs after these progestogen induced periods have been discontinued

Hypermenorrhea hypomenorrhea At least 50 per cent of endometrial biopsy or curettage specimens performed for these complaints are found to be normal proliferative normal secretory or normal menstrual endometrium if the tissue is obtained at the actual time of abnormal bleeding The latter two findings are difficult to explain unless one questions the functional capacity of the corpus luteum and its secretions The large group of patients who are found to have proliferative endometrium whether it be hypoplastic or hyperplastic are generally categorized in the group of ovulation failures and seem ideally suited for progesterone therapy In the hypermenorrhea associated with hyperplasia the following scheme has proved effective 40 mg of norethindrone or norethnodrel is given daily for 5 days This is an excellent method of producing endometrial

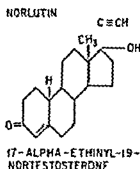


Fig 1

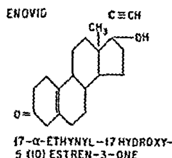


Fig 2

hemostasis although its exact mechanism is unknown It may be the result of the ability of progesterone (and presumably other progestogens) to augment estrogen metabolism converting estradiol to estrone and estriol together with inhibiting their oxidation It has been shown that estrone and estriol are proportionally greater during the luteal phase of the menstrual cycle whereas oxidation products become much reduced After 5 days of such therapy (40 mg a day) the medication is discontinued and withdrawal bleeding occasionally heavy will occur in 3-4 days Beginning on the third or fourth day of the induced flow 10 mg of norethindrone or norethnodrel is given daily for 20 days and then stopped Another withdrawal flow will then occur and this is repeated for 3 cycles Spontaneous ovulatory cycles are then awaited and a normal pattern is frequently forthcoming

In the hypoplastic proliferative pattern estrogen deficiency or end organ resistance may be etiologic Since norethnodrel contains (as a desired constituent) 1.5 per cent ethinyl estradiol 3-methyl ether no additional estrogen is needed and a plan of therapy is to administer either 10 or 20 mg daily for 20 days beginning on the third or fourth day of flow Intra-cyclic spotting may thus be avoided and artificial cycles may then be carried out as indicated above

Long Acting Preparations A new depot preparation of an esterified derivative of naturally occurring progestational hormone has recently been made available This product 17 alpha hydroxyprogester

* Available as Norlutin Parke-Davis and Co in 5 milligram tablets.

† Available as Enovid G D Searle Co in 10 milligram scored tablets (Contains 1% ethinyl estradiol 3-methyl ether)

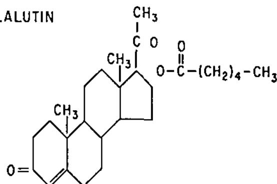


FIG 3

one caproate is a potent progestational agent which possesses a repository action of 1 2 weeks It is produced by esterification of the relatively inactive compound 17 alpha hydroxyprogesterone with caproic acid The chemical formula is given in Figure 3 A similar long acting estrogen estradiol valerate † is a companion product

In dysfunctional uterine bleeding the following *schemes have been advocated* (a) 20 mg (2 cc) of estradiol valerate as a single injection to control bleeding On the fourteenth day after the administration of estradiol valerate 250 mg of 17 alpha hydroxyprogesterone caproate and 5 mg of estradiol valerate are given in the same syringe In about two weeks bleeding will occur as a withdrawal phenomenon and the above routine is repeated giving the estradiol valerate alone on the fifth day and the combination on the fourteenth day of the artificially induced cycle Such cycles are repeated for 3 or 4 consecutive months (b) 250 mg 17 alpha hydroxy progesterone caproate is given as an initial injection and withdrawal bleeding awaited On the fourteenth day of this cycle 250 mg of 17 alpha hydroxyprogesterone caproate is given and this is repeated on the same day of the next 3 cycles

Irregular Shedding of the Endometrium

The term irregular shedding of the endometrium describes a type of dysfunctional uterine bleeding characterized by prolonged and profuse menstrual flow occurring at regular *intervals The diagnosis of this* clinical entity is established by histologic examination of endometrial tissue obtained at least five days after the onset of menstruation In normal menstrual cycles the endometrium is completely regenerated by the fifth day and microscopic examination of five day endometrium *discloses early proliferative changes* Patients with irregular shedding of the endometrium manifest incomplete regeneration of the endometrium that is the histologic characteristics of secretory endometrium are detectable as late as the fifth day of uterine bleeding The persistence of these histologic changes suggests that the onset of menstruation occurs prematurely and that some progesterone is present during the bleeding phase McKelvey and Samuels [16] demonstrated pregnanediol excretion during the bleeding phase of the menstrual cycle Holmstrom and McLennan [9] reproduced the clinical and histologic changes of irregular shedding by administering progesterone to women early in menstruation These findings suggest that the corpus luteum at the termination of its relatively uniform life span is slow to regress resulting in a continuation of the progesterone effect on the endometrium

As stated the diagnosis of irregular shedding of the endometrium can only be established if endometrial tissue is obtained for study at least five days after the onset of menstruation The necessity for proper timing undoubtedly accounts for the relatively infrequent recognition of this clinical condition If irregular shedding of the endometrium is suspected an endometrial biopsy is usually obtained at the onset of uterine bleeding Dilatation and curettage are then scheduled for the fifth or the sixth day of the flow

The administration of estrogenic substances is often useful in the treatment of irregular shedding of the endometrium Estrogenic therapy initiated at the proper time hastens the process of endometrial regeneration and decreases the intensity of *the uterine bleeding Diethylstilbestrol* 5 mgm orally each day commencing five to seven days prior to the onset of bleeding and continuing through the third or fourth day of the flow is often helpful Ethinyl estradiol 0 05 mgm twice daily by mouth in the same temporal schedule may also be employed Estrogenic therapy often diminishes the amount of uterine bleeding but may not affect the duration of the flow The endocrine treatment is not always effective and the condition may reappear after cessation of the treatment

Constant or Intermittent Uterine Bleeding

Constant or intermittent uterine bleeding is one of the most frequently encountered types of dysfunctional flow The highest incidence of grossly irregular uterine bleeding is noted in the postmenarchal and the premenopausal periods although flowing of this *nature may be manifested* at any age between the menarche and the menopause

Available as Delalutin E R Squibb & Sons supplied in vials of 5 ml each ml containing 125 mg of 17 alpha hydroxyprogesterone caproate

† Available as Delestrogen E R Squibb & Sons supplied in vials of 5 ml each ml containing 10 mg of estradiol valerate

Constant or intermittent uterine bleeding almost invariably emanates from proliferative type endometrium that is estrogen alone comprises the endometrial growth stimulus Ovulation corpus luteum formation, and progesterone secretion are not demonstrable in the majority of the cases Histologic examination of endometrial tissue from such patients discloses all degrees and gradations of estrogenic stimulation from normal proliferation to frank cystic endometrial hyperplasia depending upon the duration of the estrogenic stimulation

The pathogenesis and endocrine abnormalities responsible for anovulatory uterine bleeding will continue to remain obscure until such time as the fundamental mechanisms involved in the processes of ovulation and corpus luteum formation are elucidated An understanding of these important events must await the development of sensitive accurate and specific methods for the detection and the quantification of the pituitary gonadotropins and the ovarian steroids in body tissues and fluids The process of ovulation undoubtedly depends upon an optimum ratio between the adenohypophyseal elaboration of FSH and LH Based on this premise an imbalance between the secretion of FSH and LH provides a tenable hypothesis for the development of anovulatory cycles The precise nature of such an imbalance remains to be elucidated FSH and LH are both essential and necessary for the secretion of estrogen for this reason the absence of either of these gonadotropic hormones cannot be postulated as the *modus operandi*

The history is often helpful in determining whether constant or intermittent uterine bleeding is due to organic pathology or represents dysfunctional flowing Intermenstrual staining followed by constant or intermittent uterine bleeding most often results from intra uterine pathology Persistent or intermittent uterine bleeding commencing with a period or soon after the termination of a period usually represents dysfunctional flow Amenorrhea of several months duration often precedes constant or intermittent uterine bleeding due to hormonal imbalances Irregular uterine flowing and the absence of menstrual molimina represent two constant features of anovulatory bleeding

The diagnosis of this type of dysfunctional flowing is established by examining the endometrium obtained at the onset of bleeding or during the bleeding phase Endometrial tissue recovered at this time will disclose either proliferation or hyperplasia that is evidence of progestational activity is lacking Although the endometrial biopsy technic provides the clinician with a convenient method for obtaining tissue for histologic study and diagnosis curettage is usually indicated As a general rule dilatation and curettage should be performed on all patients who present themselves with constant or intermittent uterine bleeding An exception to this general policy relates to patients in the postmenarchal period In these young girls the presumptive diagnosis of anovulatory uterine bleeding can be established on the basis of the history alone without resorting to the endometrial biopsy technic or to curettage

The clinical management of patients with constant or intermittent uterine flowing is directed toward the disclosure of existing organic pathology and the cessation of bleeding Endometrial tissue is obtained at the onset of uterine bleeding or during the bleeding period The endometrial biopsy technic is employed for this purpose The patient is then scheduled for dilatation and curettage usually a few days hence during which time histologic examination of the tissue is completed Temporary cessation of the bleeding will result from the curettage in the majority of the patients If the existence of organic pathology is eliminated as the cause of the abnormal bleeding cyclic therapy with progesterone or estrogen and progesterone represents an effective method of treatment Details of the therapy with progesterone and estrogen and progesterone have been outlined in the section of this chapter devoted to profuse or prolonged uterine bleeding

FUNCTIONING OVARIAN TUMORS

Granulosa-cell Tumors

Granulosa-cell tumors comprise about 3 per cent of all ovarian neoplasms and are the most frequently encountered functioning ovarian tumor Although granulosa-cell tumors have been observed at various ages (17 months to 84 years) their occurrence is rare before 30 and after 65 years of age A large number of these tumors has been reported in post menopausal women for this reason the interval between 45 and 65 years of age seems to be singularly predisposed to the genesis of this tumor Histologically the cells of the tumor are epithelial in appearance not unlike the cells of the granulosa layer of the mature graafian follicle These cells are remarkably uniform with indistinct cell outlines and granular cytoplasm The nuclei are round to ovoid centrally placed and usually sharply delineated from the cytoplasm Several cellular patterns have been described depending upon the arrangement of these epithelial elements in the connective tissue matrix Thus they may be folliculoid adenomatoid cylindroid or sarcomatoid

About 90 to 95 per cent of granulosa-cell tumors are unilateral and vary in size from a millimeter or less to 20 centimeters They are usually encapsulated rarely pedunculated and do not adhere to surrounding structures in most instances These tumors tend

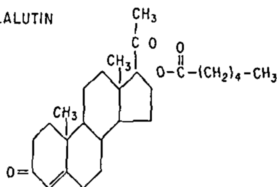


FIG 3

one caproate* is a potent progestational agent which possesses a repository action of 1 2 weeks It is produced by esterification of the relatively inactive compound 17 alpha hydroxyprogesterone with caproic acid The chemical formula is given in Figure 3 A similar long acting estrogen estradiol valerate † is a companion product

In dysfunctional uterine bleeding the following schemes have been advocated (a) 20 mg (2 cc) of estradiol valerate as a single injection to control bleeding On the fourteenth day after the administration of estradiol valerate 250 mg of 17 alpha hydroxyprogesterone caproate and 5 mg of estradiol valerate are given in the same syringe In about two weeks bleeding will occur as a withdrawal phenomenon and the above routine is repeated giving the estradiol valerate alone on the fifth day and the combination on the fourteenth day of the artificially induced cycle Such cycles are repeated for 3 or 4 consecutive months (b) 250 mg 17 alpha hydroxyprogesterone caproate is given as an initial injection and withdrawal bleeding awaited On the fourteenth day of this cycle 250 mg of 17 alpha hydroxyprogesterone caproate is given and this is repeated on the same day of the next 3 cycles

Irregular Shedding of the Endometrium

The term irregular shedding of the endometrium describes a type of dysfunctional uterine bleeding characterized by prolonged and profuse menstrual flow occurring at regular intervals The diagnosis of this clinical entity is established by histologic examination of endometrial tissue obtained at least five days after the onset of menstruation In normal menstrual cycles the endometrium is completely regenerated by the fifth day and microscopic examination of five day endometrium discloses early proliferative changes Patients with irregular shedding of the endometrium manifest incomplete regeneration of the endometrium that is the histologic characteristics of secretory endometrium are detectable as late as the fifth day of uterine bleeding The persistence of these histologic changes suggests that the onset of menstruation occurs prematurely and that some progesterone is present during the bleeding phase McKelvey and Samuels[16] demonstrated pregnanediol excretion during the bleeding phase of the menstrual cycle Holmstrom and McLennan[9] reproduced the clinical and histologic changes of irregular shedding by administering progesterone to women early in menstruation These findings suggest that the corpus luteum at the termination of its relatively uniform life span is slow to regress resulting in a continuation of the progesterone effect on the endometrium

As stated the diagnosis of irregular shedding of the endometrium can only be established if endometrial tissue is obtained for study at least five days after the onset of menstruation The necessity for proper timing undoubtedly accounts for the relatively infrequent recognition of this clinical condition If irregular shedding of the endometrium is suspected an endometrial biopsy is usually obtained at the onset of uterine bleeding Dilatation and curettage are then scheduled for the fifth or the sixth day of the flow

The administration of estrogenic substances is often useful in the treatment of irregular shedding of the endometrium Estrogenic therapy initiated at the proper time hastens the process of endometrial regeneration and decreases the intensity of the uterine bleeding Diethylstilbestrol 5 mgm orally each day commencing five to seven days prior to the onset of bleeding and continuing through the third or fourth day of the flow is often helpful Ethinyl estradiol 0 05 mgm twice daily by mouth in the same temporal schedule may also be employed Estrogenic therapy often diminishes the amount of uterine bleeding but may not affect the duration of the flow The endocrine treatment is not always effective and the condition may reappear after cessation of the treatment

Constant or Intermittent Uterine Bleeding

Constant or intermittent uterine bleeding is one of the most frequently encountered types of dysfunctional flow The highest incidence of grossly irregular uterine bleeding is noted in the postmenarchal and the premenopausal periods although flowing of this nature may be manifested at any age between the menarche and the menopause

* Available as Delalutin E R Squibb & Sons supplied in vials of 5 ml each ml containing 125 mg of 17 alpha hydroxyprogesterone caproate

† Available as Delestrogen E R Squibb & Sons supplied in vials of 5 ml each ml containing 10 mg of estradiol valerate

Constant or intermittent uterine bleeding almost invariably emanates from proliferative type endometrium that is estrogen alone comprises the endometrial growth stimulus Ovulation corpus luteum formation and progesterone secretion are not demonstrable in the majority of the cases Histologic examination of endometrial tissue from such patients discloses all degrees and gradations of estrogenic stimulation from normal proliferation to frank cystic endometrial hyperplasia depending upon the duration of the estrogenic stimulation

The pathogenesis and endocrine abnormalities responsible for anovulatory uterine bleeding will continue to remain obscure until such time as the fundamental mechanisms involved in the processes of ovulation and corpus luteum formation are elucidated An understanding of these important events must await the development of sensitive accurate and specific methods for the detection and the quantification of the pituitary gonadotropins and the ovarian steroids in body tissues and fluids The process of ovulation undoubtedly depends upon an optimum ratio between the adenohypophyseal elaboration of FSH and LH Based on this premise an imbalance between the secretion of FSH and LH provides a tenable hypothesis for the development of anovulatory cycles The precise nature of such an imbalance remains to be elucidated FSH and LH are both essential and necessary for the secretion of estrogen for this reason the absence of either of these gonadotropic hormones cannot be postulated as the *modus operandi*

The history is often helpful in determining whether constant or intermittent uterine bleeding is due to organic pathology or represents dysfunctional flowing Intermenstrual staining followed by constant or intermittent uterine bleeding most often results from intra uterine pathology Persistent or intermittent uterine bleeding commencing with a period or soon after the termination of a period usually represents dysfunctional flow Amenorrhea of several months duration often precedes constant or intermittent uterine bleeding due to hormonal imbalances Irregular uterine flowing and the absence of menstrual molimina represent two constant features of anovulatory bleeding

The diagnosis of this type of dysfunctional flowing is established by examining the endometrium obtained at the onset of bleeding or during the bleeding phase Endometrial tissue recovered at this time will disclose either proliferation or hyperplasia that is evidence of progestational activity is lacking Although the endometrial biopsy technic provides the clinician with a convenient method for obtaining tissue for histologic study and diagnosis curettage is usually indicated As a general rule dilatation and curettage should be performed on all patients who present themselves with constant or intermittent uterine bleeding An exception to this general policy relates to patients in the postmenarchal period In these young girls the presumptive diagnosis of anovulatory uterine bleeding can be established on the basis of the history alone without resorting to the endometrial biopsy technic or to curettage

The clinical management of patients with constant or intermittent uterine flowing is directed toward the disclosure of existing organic pathology and the cessation of bleeding Endometrial tissue is obtained at the onset of uterine bleeding or during the bleeding period The endometrial biopsy technic is employed for this purpose The patient is then scheduled for dilatation and curettage usually a few days hence during which time histologic examination of the tissue is completed Temporary cessation of the bleeding will result from the curettage in the majority of the patients If the existence of organic pathology is eliminated as the cause of the abnormal bleeding cyclic therapy with progesterone or estrogen and progesterone represents an effective method of treatment Details of the therapy with progesterone and estrogen and progesterone have been outlined in the section of this chapter devoted to profuse or prolonged uterine bleeding

FUNCTIONING OVARIAN TUMORS

Granulosa-cell Tumors

Granulosa-cell tumors comprise about 3 per cent of all ovarian neoplasms and are the most frequently encountered functioning ovarian tumor Although granulosa cell tumors have been observed at various ages (17 months to 84 years) their occurrence is rare before 30 and after 65 years of age A large number of these tumors has been reported in post menopausal women for this reason the interval between 45 and 65 years of age seems to be singularly predisposed to the genesis of this tumor Histologically the cells of the tumor are epithelial in appearance not unlike the cells of the granulosa layer of the mature graafian follicle These cells are remarkably uniform with indistinct cell outlines and granular cytoplasm The nuclei are round to ovoid centrally placed and usually sharply delineated from the cytoplasm Several cellular patterns have been described depending upon the arrangement of these epithelial elements in the connective tissue matrix Thus they may be folliculoid adenomatoid cylindroid or sarcomatoid

About 90 to 95 per cent of granulosa cell tumors are unilateral and vary in size from a millimeter or less to 20 centimeters They are usually encapsulated rarely pedunculated and do not adhere to surrounding structures in most instances These tumors tend

to be solid although cystic spaces are occasionally noted The cut surface appears gray pink to yellow and has a homogeneous granular appearance Hemorrhage and necrosis may be prominent since the tumors are very vascular

Various theories relating to the histogenesis of granulosa cell tumors have been advanced in the last three decades Robert Meyer[11] postulated that these tumors developed from embryonic rests of granulosa cells under the influence of some unusual stimulus Novak[16] suggested that they originate from undifferentiated ovarian mesenchyme Robinson promulgated the hypothesis that the graafian follicle itself underwent rapid growth to the development of typical folliculomas Parkes *et al*[9] irradiated mice and noted the degeneration of ova and follicles followed by a replacement of interfollicular tissue by luteal like cords with a secondary proliferation of folliculoid cells McKay Hertig and Hickey[14] have postulated that granulosa cell tumors have their origin in atretic follicles which contain persistent granulosa cells McKay Robinson and Hertig[15] previously had shown that the cells of the theca interna probably produce the estrogenic steroids in tumors as well as in normal ovaries Therefore symptoms resulting from excessive estrogenic secretion depend *not on the amount or distribution of granulosa cells* but upon the amount and functional capacity of the theca interna cells

The symptomatology resulting from granulosa cell tumors depends upon the age at which estrogen secretion occurs In children precocious puberty is noted with development of the secondary sex characteristics and the initiation of uterine bleeding During the reproductive phase amenorrhea may be manifested Profuse and continuous uterine bleeding often follows these periods of amenorrhea Post menopausally regular or irregular uterine bleeding is noted

Endometrial hyperplasia resulting from constant estrogenic stimulation is said to occur in about 70 per cent of patients with functioning granulosa cell tumors Endometrial carcinoma leiomyomata breast lesions and cortical stromal hyperplasia of the opposite ovary occur with more frequency than would be expected by chance alone Malignancy frequently occurs in these tumors in older women but is only rarely noted in younger women For this reason conservative surgery is recommended for younger individuals A few tumors will be obviously malignant upon histologic examination with irregularly arranged hyperchromatic cells bearing little resemblance to granulosa cells If capsular invasion or extension to adjacent structures is noted treatment should include *total hysterectomy* bilateral salpingo oophorectomy and removal of all involved organs Surgery should then be followed with a full course of *deep x ray therapy*

The recurrence rate of so called benign granulosa cell tumors is said to approximate 30 per cent Such recurrences may occur 15 20 years postoperatively but they are most frequently noted 2 5 years after surgery

Thecomas

Thecomas are much less common than granulosa cell tumors Thecomas constitute 1 2 per cent of all ovarian tumors and 3 5 per cent of solid ovarian tumors Such tumors have been reported in females from 1 to 90 years of age but 70 per cent occur in the postmenopausal group Thecomas are unilateral and usually are benign They vary in size from microscopic cellular aggregations to ovarian masses measuring 15 20 cm The larger tumors are often adherent to surrounding structures The cut surface is either homogeneously yellow or manifests a somewhat fibrotic appearance which is diffusely mottled with yellow coloration Hyalinization or liquefaction may be present Histologically a thecoma resembles a fibroma with plump fusiform cells arranged in a whorled pattern In contradistinction to fibromas however intracellular fat globules may be *demonstrated with fat stains*

There is reason to believe that the histogenesis of thecomas differs from that of granulosa cell tumors Woll *et al*[2] noted a change in the aging ovary which they designated as cortical stromal hyperplasia This consists of a diffuse or nodular thickening of the ovarian cortex which is unusually dense and often assumes the shape of spherical or scalloped masses There is usually a diminution in the number of ova and follicles and the stromal cells are grouped into more than the usual number of whorls and fascicles Intercellular collagen is scant accounting for the fact that the cortex appears an unusually dark blue when the stained section is viewed by the unaided eye Such cortical thickening varies from patient to patient in some ovaries it occurs only focally producing nodular masses These focal areas are often arranged in whorls and are identical with the histologic pattern of thecomas The evidence then that theca cell tumors have their origin in cortical stromal hyperplasia is as follows (a) the histologic pattern of both tissues is identical including the presence of hyaline placques (b) transitional stages between cortical stromal hyperplasis and thecomas are observed (c) patients with theca cell tumors of one ovary almost invariably have cortical stromal hyperplasia of the opposite uninvolved ovary and (d) both conditions occur primarily in the postmenopausal age groups

The clinical picture with thecomas is not always clearly one of estrogen secretion Although patients

with thecomas often manifest anovulatory uterine bleeding persistent flowing from secretory endometrium has been noted to accompany bilateral thecomas in rare instances The most characteristic clinical feature is usually atypical uterine bleeding similar to that found with granulosa cell tumors Enlargement of the breasts is occasionally noted The uterus even in the postmenopausal period is found to be enlarged and softened and the endometrium is typically hyperplastic in nature

Since the large majority of thecomas are benign conservative surgery is indicated Meigs syndrome may be associated with benign thecomas especially if there is torsion of the ovarian mass In 1955 the seventh malignant thecoma was reported by Jew and Gross [11] in a 39 year old nulliparous patient In this case there was capsular invasion with metastases to the omentum and small bowel mesentery Histologically pleomorphic cell structure necrosis excessive mitoses and invasion of ovarian serosa were noted The omental metastases disclosed the same histologic picture Endocrine dysfunction did not result from this tumor for the patient manifested no aberrations of menstrual function In the presence of obvious malignancy the treatment includes complete hysterectomy bilateral salpingo-oophorectomy and postoperative irradiation

Luteoma

Most observers contend that the ovarian neoplasm classified as a luteoma is actually a luteinized granulosa cell tumor since in at least a small group of patients progesterone effects upon the endometrium have been demonstrated The luteoma is a rare tumor and as such should be distinguished from the virilizing lipoid cell tumor (adrenal rest tumor) of the ovary Luteomas are unilateral and benign Histologically the cells of the tumor are large with pale staining cytoplasm and well defined cell boundaries A sufficient number of cases of luteomas has not been reported in the medical literature to allow speculation concerning the incidence the symptomatology or the malignant potential of the tumor Simple surgical excision in most cases is deemed adequate therapy

Hyperthecosis Syndrome

Shippel [2] has recently postulated the intriguing theory that the pure thecoma is a masculinizing tumor This hypothesis is the result of an analysis of the clinical histories and pathologic specimens from 37 patients However as a result of admixturing with granulosa cells the tumor may produce endometrial patterns ranging from cystic glandular hyperplasia to complete atrophy and menstrual abnormalities from dysfunctional flow to amenorrhea It is contended that the hyperthecosis syndrome comprises six pathologic phases the first three being concerned with thecal granulosal synergism and the last three with thecal dominance Thus if untreated patients manifest various phases of irregular uterine bleeding progressing eventually to amenorrhea Pathologically the endometrium shows first crowding of glands glandular intussusception cystic glandular hyperplasia and finally atrophy The female is transformed into a masculinized hirsute sterile often frigid individual with temporal hair recession atrophied uterus and endometrium and occasionally clitorine enlargement and breast atrophy

Attractive as the theory above may be it does not completely explain the feminizing potentials of theca cell tumors in which very few (if any) granulosa cells are present for synergism and in which ketosteroid substances are demonstrable in the theca cell

Arrhenoblastoma

The arrhenoblastoma is a rare ovarian tumor which characteristically causes amenorrhea defeminization and virilization These tumors vary in size from 0 5 cm to 15 cm although much larger neoplasms of this type have been reported Arrhenoblastomas are usually unilateral The surface of the tumor is smooth but occasionally lobulated the tumor may be solid or cystic upon cutting and demonstrates a yellowish or tan hue Histologically three variants are recognized (1) typical tubular form (tubular adenoma of Pick) (2) atypical more solid form (intermediate) and (3) undifferentiated form (sarcomatoid) Although the age at which these tumors have been noted ranges from fifteen to fifty years arrhenoblastomas are characteristically tumors of young women the majority of cases occurring between twenty and thirty years of age

Arrhenoblastomas are thought to arise from ambivalent cellular elements in the ovary which tend toward the masculine cell type in their development producing a hormone which activates both physical and psychic latent heterosexual characteristics The possibility of origin from the testicular element of an ovotestis has been entertained About 100 arrhenoblastomas have been reported in the world literature The over all incidence of clinical malignancy approximates 20 per cent in those cases in which adequate follow up studies were possible The malignancy is usually evident at the time of operation since in many cases there is extension or invasion of adjacent tissues In those patients in whom the tumor is freely movable nonadherent and limited to one ovary the prognosis is excellent The extent of surgical treatment therefore must be determined by the intra abdominal findings

Virilizing Lipoid Cell Ovarian Tumors

In this group may be included the diverse and inadequately studied tumors variously designated as luteinomas adrenal rest tumors hypernephromas interstitiomas and masculinovoblastomas These neoplasms are usually well encapsulated round or oval in shape and measure from 2 cm to 16 cm in diameter Cystic changes occasionally have been noted The orange yellow color noted upon cutting the tumors is due to the presence of lipids within the cytoplasm of the large polyhedral cells The histologic similarity between adrenal rest tumors luteomas and luteinized granulosa or theca cell tumors is so striking that careful evaluation of the patient s clinical signs is necessary for accurate classification

Symptomatology includes progressive defeminization deepening of the voice hirsutism scanty uterine bleeding amenorrhea and clitoric enlargement Occasionally twisting of the cyst brings the patient to surgery before the symptoms are pronounced The urinary excretion of 17 ketosteroids is usually elevated and FSH diminished

Fractionation studies of 17 ketosteroids may be of some value in differential diagnosis since it has been found that in virilizing adrenal gland tumors (arising in the adrenal itself) the *beta* fraction of the elevated 17 ketosteroids is itself elevated This is easily detected by the blue color reaction of Allen (J Clin Endocrinology 10 54 70 1950) which measures urinary dehydroisoandrosterone a beta 17 keto steroid which is normally present in amounts too small to be detected by the Allen test In two patients with adrenal like tumors arising in the ovary the beta fraction of 17 ketosteroids has not been elevated (Paterson and Schamberger—Acta Endocrinol 13 109 22 1953 Obst & Gynec Survey 12 774 1957—ed note)

Microscopically the tumors consist of large polyhedral cells suggestive of lipoid containing cells or adrenal cortical cells with deeply staining granular nuclei The cells are arranged in strands or columns with a fine reticular stroma between the columns An abundance of fat is noted when the tumors are subjected to Sudan III stains

These cells resemble the cells of the adrenal cortex so closely that an adrenal genesis has been postulated In view of the very intimate embryological relationship of the adrenal cortex and the ovarian medulla this opinion becomes quite tenable However the histologic appearance of constituent cells is not an infallible criterion of their origin especially since certain other cell types such as lutein cells or the interstitial cells of Leydig also closely resemble adrenal cells

Treatment of these tumors is surgical with unilateral oophorectomy usually being sufficient to bring about reversion to femininity

Ovarian Hilus Cell (Leydig Cell) Tumors

Hyperplasia of the rather frequently occurring 'hilus cells of the normal ovary has recently been associated with masculinization syndromes in females Berger [3] and Sternberg [4] have described certain disorders due to abnormalities of these cells The aggregates are probably analogous to the male Leydig cells and show typical Reinke crystalloids by the Masson trichrome stain However hilar cell rests may be more common in normal ovaries than has hitherto been appreciated The authors have identified them on numerous occasions in ovaries removed from women who showed no stigmata of masculinization There is no doubt that upon occasion they may undergo hyperplasia or tumor formation with subsequent virilization Being Leydig like cells they are under the influence of pituitary or pituitary like hormones

In cases of obvious masculinization the physician should not be misled from the possibility of adrenal tumors or adrenal hyperplasia even in the presence of such ovarian hilar changes

Husslein [10] has reported five cases of hilus cell tumor in elderly women accompanied by feminization This investigator suggests that there are two types of hilus cells one masculinizing and one feminizing

Struma of the Ovary

On rare occasions a benign cystic teratoma (dermoid) contains sufficient functioning thyroid tissue to produce hyperthyroidism About twelve of these cases have been reported in the medical literature Almost all benign cystic teratomas contain small amounts of thyroid tissue but the term *struma ovarii* should be limited to tumors in which thyroid tissue is either the sole constituent of or constitutes the major part of the neoplasm

The tumor varies considerably in size from tiny nodules to neoplasms measuring 15 20 cm The surface of the neoplasm is nodular and encapsulated Upon sectioning the tumor the color is a waxy brownish shade resembling thyroid the cut section is honeycombed Histologically the alveoli may occasionally show papillary projections which suggest hyperplasia In rare instances the appearance may be that of a malignant struma with capsular perforation and invasion of the surrounding tissue

Treatment consists of operative removal of the neoplasm and if malignant complete hysterectomy and bilateral salpingo oophorectomy is indicated

Henriksen[7] has recently reported a case of struma salpingii which was in effect a dermoid tumor of the oviduct consisting entirely of adult thyroid tissue

Ovarian Agenesis

This condition is known variously as Turner's syndrome or ovarian dwarfism The classical clinical features are (a) sexual infantilism (b) retarded growth and development (c) scanty distribution of pubic and axillary hair and (d) delayed union of the epiphyses and diffuse osteoporosis About 100 cases have been reported to date In those patients in whom surgical exploration was performed the usual finding was bilateral congenital absence or stunting of the ovaries When present the ovaries appeared as white or gray strands or mounds of tissue in the peritoneal fold lateral to the broad ligament Microscopically total absence of the germinal elements (primordial follicles and ova) is noted but the ovarian stroma is recognizable

While the diagnosis may be suggested by the characteristic clinical manifestations of the disease certain laboratory determinations are of considerable value The urinary excretion of 17 ketosteroids is low and as would be expected with primary ovarian failure the concentration of pituitary gonadotropins in the urine is high

However the increased urinary FSH does not become elevated until puberty and cases have been reported with normal titers after puberty The chromosomal sex pattern as determined by oral mucosal smears (J A M A 161 683 1956) skin biopsy (Lancet 269 67 1955) or blood smears (J Clin Endocrinology 7 385 1947) is usually *male* The finding of a female chromatin pattern does not exclude the diagnosis If every patient with primary amenorrhea especially those with short stature were studied from the standpoint of genetic sex regardless of the presence or absence of other anomalies it is probable that many more cases of gonadal dysgenesis would be correctly diagnosed

The pathogenesis of this syndrome remains obscure although the short stature and ovarian maldevelopment are believed to be linked genetic defects The large number of congenital anomalies found associated with this disease lends credence to this thought Developmental anomalies often associated with Turner's syndrome include webbing of the neck mental retardation ocular abnormalities (cataracts strabismus coloboma and tubular vision) deafness vascular abnormalities such as coarctation of the aorta bony abnormalities (pes cavus spina bifida shortened phalanges and Madelung's deformity) renal abnormalities and unexplained hypertension

The sexual infantilism may be ascribed to the low estrogenic titer since the internal and external genitalia are not usually deformed and grow under the stimulus of exogenous estrogen

The sparse amount of axillary and pubic hair may result from diminished 17 ketosteroid precursors When estrogen is administered to these patients hair growth increases as does the excretion of 17 ketosteroids in the urine

Despite the elevated gonadotropic levels vasomotor symptoms are not manifested Treatment of this condition consists of the administration of estrogenic preparations to stimulate development of the secondary sex characteristics The patient may thus regain a normal feminine appearance and uterine bleeding may be initiated by cyclic endocrine therapy Details of this therapy have been presented in the section of this chapter devoted to delayed adolescent development and delayed menarche

Polycystic Ovaries

This derangement of the ovaries is frequently noted in women whose ovulatory processes are faulty In addition to anovulatory cycles patients with polycystic ovaries also manifest secondary amenorrhea male type hirsutism infertility and uterine hypoplasia These clinical signs were originally described by Irving Stein and are now commonly known as the Stein Leventhal syndrome Polycystic ovaries are commonly noted in women 17 30 years of age In a typical case the presumptive diagnosis of Stein Leventhal syndrome can often be established from the history alone Puberty usually occurs normally and at the expected time Initially uterine bleeding may be regular or irregular A few years hence periods of amenorrhea progressively longer in duration are noted Not infrequently the amenorrhea becomes permanent Hirsutism varies in amount from patient to patient Sterility naturally results from this disease entity

Physical examination in early cases of the Stein Leventhal syndrome often reveals no significant somatic changes However patients with long standing amenorrhea and marked hirsutism manifest male somatic changes The breasts are usually small and the distribution of pubic hair resembles that of the male the external genitalia are normal Pelvic examination usually discloses a hypoplastic uterus The ovaries are palpably enlarged in only 50 per cent of the cases If the ovaries are enlarged they rarely are larger than 6 cm in diameter Pneumoroentgenography has been suggested as a diagnostic procedure to corroborate a presumptive diagnosis of Stein Leventhal syndrome Culdoscopy may also be helpful in the diagnosis of this condition

Following the establishment of a definite diag

nosis bilateral ovarian wedge resection has been suggested as the treatment of choice. Stein has reported on 90 patients treated by wedge resection [5]. Sixty four of these women complained of infertility. Eighty nine per cent subsequently became pregnant; recurrence of the polycystic condition did not ensue in any of the patients.

Unfortunately this diagnosis has been applied by many gynecologists to various fringe conditions in which ovulation does not occur and in whom slight ovarian cortical fibrosis is found at the time of surgery. Bilateral wedge resection in these patients is uniformly unsuccessful.

PREMATURE OVARIAN SENILITY

Spontaneous premature menopause is uncommon but occasionally may follow bouts of severe infection, prolonged lactation, or severe debilitating diseases. Hypo ovarianism should be considered in patients who do not begin to menstruate until 18 or 19 years of age, bleed irregularly for 8 9 years and then have complete cessation of uterine bleeding at 30 years of age. This ovarian atrophy may be the result of ripening and atresia of an excessive number of follicles during menstrual cycles or to a congenitally insufficient number of primordial follicles.

The diagnosis is suspected when a history of secondary amenorrhea coupled with vasomotor symptoms is obtained. The titer of gonadotropic substances in the urine is elevated in this condition.

THE MENOPAUSE

Between the ages of 45 and 55 there occur in the ovary a depletion in the number of follicles and a reduction in the stroma and the vascularity of the organ. During this period irregularities of menstruation and characteristic vasomotor symptoms are noted.

The basic endocrine pattern of the menopause is one of ovarian insufficiency. It is of considerable interest that measurable amounts of estrogen, presumably from the adrenal cortex, continue to be excreted in the urine long after cessation of ovarian function. Recent studies of urinary steroid excretion indicate a decline in total estrogen excretion from the third to the sixth decade with a constant low level thereafter.

Pituitary gland enlargement with an increase in cellular elements follows the gradual decline of estrogenic activity. The follicle stimulating hormone increases in concentration whereas the titer of the luteinizing hormone may increase or decrease. The excessive secretion of FSH may be diminished by administering adequate amounts of estrogen whereas administration of the same amount of estrogen may actually increase the LH output.

As estrogenic secretory activity diminishes the uterus and the cervix decrease in size. The endometrium becomes atrophic and the vaginal epithelium thins and contains little glycogen. The ovarian hilar vessels become sclerosed and the ovarian cortex thins. The anabolic effect of the estrogens is lost whereas adrenal corticosteroids continue to be secreted at almost a normal level. The over all effect according to Albright [1] is a gradual attrition of skin and muscles with the development of osteoporosis.

Psychological problems during the menopause are frequently encountered. Numerous studies have indicated that the symptoms of anxiety and depression can be related to pre existing emotional disorders which are exaggerated by the situational stress occurring at the time of the menopause.

Important recent advances in the study of the etiology of atherosclerosis have demonstrated the importance of estrogen in maintaining homeostasis in the cardiovascular system of the human female. Wuest [2] and others have demonstrated that the degree of arteriosclerosis in women subjected to bilateral oophorectomy is greater than that in women with intact ovaries. Other investigators have shown that in cholesterol fed chicks estrogens can inhibit the development of coronary atherosclerosis and can cause regression of established atherosclerosis. This favors prolonged administration of estrogens in the postmenopausal period, a rationale that several years ago was looked upon with trepidation because of the possible relationship to endometrial and/or mammary carcinoma.

Any variation of abnormal bleeding may occur at the menopause. It is widely stated that 50 per cent of women who bleed postmenopausally will be found to have cancer somewhere in the genital tract. For this reason uterine curettage is mandatory when postmenopausal bleeding arises.

The proper management of the menopausal patient depends upon whether the presenting complaints and symptoms are due to estrogen deficiency or to anxiety and depression. Symptoms arising from anxiety states may be effectively managed by sympathetic listening, simple explanations and reassurance. Small doses of barbiturates are occasionally indicated but should be gradually withdrawn as the symptoms subside. If the major symptoms are those of vasomotor instability, that is hot flushes and sweats, beneficial results may be expected from the proper use of estrogenic preparations. In most cases small doses of an oral estrogenic preparation will afford satisfactory relief. When estrogens are employed they must be administered according to a definite schedule. In general the dosage should be kept to a minimum since it is undesirable to induce

uterine bleeding of any degree Diethylstilbestrol 0 25 mgm or ethinyl estradiol 0 02 mgm may be given daily for three weeks with interruption of the therapy for one week Recent reports concerning the use of a long acting fat stored estrogen chlorotriani sene (TACE Merrell) have been encouraging Us ing this estrogenic preparation the therapy may be interrupted for longer intervals and the patient will remain asymptomatic as long as sufficient estrogen is being released from body fat

Although there is no direct evidence that estro gens are carcinogenic in humans numerous inves tigators have emphasized suggestive relationships The problem has recently been reviewed by Novak [19] who concludes that although estrogen administration can produce endometrial hyperplasia and microscopic patterns indistinguishable from early endometrial carcinoma sufficient evidence to conclude that estro gens definitely cause adenocarcinoma of the uterus has not been accumulated

CARCINOMA OF THE BREAST AND THE OVARY

In 1896 Beatson performed ovariectomy for dis seminated carcinoma of the breast and obtained a remission of the disease In 1905 Lett [3] reported a series of 99 patients with inoperable breast cancer treated by oophorectomy In this series he observed that a good clinical response occurred in 41 per cent of the patients younger than 50 years of age but in only 23 per cent of those over 50 These figures represent the number of breast cancers believed to be hormonally dependent

These early observations were given added sup port in 1941 by the investigations of Pincus and Graubard [1] who demonstrated that postmenopausal *women with mammary carcinoma excreted more* estrogens in their urine than normal postmenopausal women The cause of this increase in endogenous estrogen was suggested recently by the observations of Sommers [4] who studied the ovaries of women dying of carcinoma of the breast He found that ovarian cortical stromal hyperplasia occurred in 86 per cent of these cancer patients but in only 37 per cent of the controls Furthermore he noted that 80 per cent of patients over 80 years of age who died of breast cancer manifested ovarian cortical stromal hyperplasia

Thus a definite ovarian hormonologic correlation exists in about half of all mammary cancers Clinical and laboratory tests are available to aid in differen tiating those tumors which are dependent from those which seem to have autonomous growth potential At the present time it is not known whether pro phylactic castration at the time of radical mastectomy will affect the five- and ten year survival rates of patients whose tumors have spread to adjacent lymph nodes There is no doubt however that in the presence of distant osseous metastases castration will provide palliation in hormonally dependent tumors

In some postmenopausal women the administra tion of estrogenic substances will induce remissions of metastatic disease In others it may stimulate the growth of the localized cancer The mechanism by which estrogens induce remissions in women with breast cancer has not been elucidated Estrogens may induce beneficial effects by the suppression of pituitary function

BIBLIOGRAPHY

1 Albright F 1947 Osteoporosis Ann Int Med 27 861 82
2 Beatson G T 1896 On the treatment of inoperable cases of carcinoma of the mamma suggestions for a new method of treatment with illustrative cases Lancet 2 104 7
3 Berger L 1942 Tumeur des cellules sympatheco tropes de l ovaire avec virilisation Rev Canad Biol 1 539 66
4 Brown R W Woodbury D M and Sayers G 1952 Inhibition of thyrotropin gonadotropins and prolactin by ACTH J Clin Endocrin & Metab 12 939 40
5 Graves W P 1930 *Some observations on etiology* of dysfunctional uterine bleeding Am J Obst & Gynec 20 500 518
6 Harris G W 1948 Neural control of the pituitary gland Physiol Rev 28 139 79
7 Henriksen E 1955 Struma salpingii report of a case Obst & Gynec 5 833 5
8 Holmstrom E G and Brown R W 1956 Effect of prolactin on the normal human menstrual cycle Obst & Gynec 8 203 6
9 Holmstrom E G and McLennan C E 1947 Men orrhagia associated with irregular shedding of the endometrium Am J Obst & Gynec 53 727 48
10 Husslein H 1952 Fonction endo rine du hilus cell adenome de l ovaire Compt rend soc franc gynec 22 55 7
11 Jew E W Jr and Gross P 1955 Malignant the coma with metastases Am J Obst & Gynec 69 857 60
12 Jones G E S Howard J E and Lan ford H 1953 Use of cortisone in follicular phase disturb ance Fertility & Sterility 4 49 62
13 Lett H 1905 An analysis of 99 cases of inoperable carcinoma of the breast treated by oophorectomy Lancet 1 227 8
14 McKay D G Hertig A T and Hickey W F 1953 The histogenesis of granulosa and theca cell tumors of the human ovary Ob t & Gynec 1 125 36
15 McKay D G Robinson D and Hertig A T 1949 Histochemical observations on granulosa cell tumors thecomas and fibromas of ovary Am J Obst & Gynec 58 625 39
16 McKelvey J L and Samuels L T 1947 Irregular shedding of the endometrium Am J Obst & Gynec 53 627 36
17 Meyer R 1931 Pathology of some pecial ovarian tumors and their relation to sex characteristics Am J Obst & Gynec __ 697 713

18 Novak E 1942 Endocrine effects of certain dyson togenetic tumors of the ovary Endocrinology 30 953 8
19 Novak E R 1954 Relationship of endometrial hy perplasia and adenocarcinoma of the uterine fundus J A M A 154 217 20
20 Parkes A S Brambell E W R and Fielding U 1927 Ovarian regeneration in the mouse after com plete double ovariotomy Proc Royal Soc 101 328 54
21 Pincus G and Graubard M 1940 Estrogen me tabolism in cancerous and non cancerous women Endocrinology 26 427 32
22 Robinson M R 1930 Primary and secondary ova rian cancer a histogenetic morphological and clin ical study Surg Gynec & Obst 51 321 44
23 Shippel S 1955 The ovarian theca cell The hy perthecosis syndrome J Obst & Gynaec Brit Emp LXII No 3 321 53 (June)
24 Sommers S C and Teloh H 1952 Ovarian stromal hyperplasia in breast cancer Arch Path 53 160-66
25 Stein I F 1955 The Stein Leventhal syndrome West J Surg 63 319 23
26 Sternberg W H Segaloff A and Gaskill C J 1953 Influence of chorionic gonadotropin on human ovarian hilus cells J Clin Endocrin & Metab 13 139 53
27 Woll E Hertig A T Smith G V and Johnson L C 1948 The ovary in endometrial carcinoma Am J Obst & Gynec 56 617 33
28 Wuest J H Jr Dry T J and Edwards J E 1953 The degree of coronary atherosclerosis in bi laterally oophorectomized women Circulation 7 801 9

VIII

Clinical Concepts of Infertility

LUIGI MASTROIANNI, JR., M.D.

IN THE LAST quarter century there has been a renaissance in thinking in the management of human reproductive failure. Though the importance of this area is perhaps better expressed in terms of its effect on the lives of the individuals involved, it has been frequently stated that at least 10 per cent of couples are involuntarily infertile. It is now generally agreed that an infertility investigation is in order if conception fails to occur after a year's exposure. Statistical studies as to what constitutes normal fertility support this view.[1, 5, 160] Minimal standards for the investigation of infertility have been recommended by the American Society for the Study of Sterility and include evaluation of the seminal, tubal, cervical and ovarian factors.[9] Diagnostic techniques have been developed which are useful in such an investigation. As in other intricate fields of medical endeavor, therapy has lagged behind diagnosis. The clinician has been presented with a plethora of new therapeutic approaches, many of which have proved disappointing. We propose to review the diagnostic and therapeutic methods presently available with emphasis on the clinical approach to the problem of human infertility.

EVALUATION OF THE MALE ROLE

Infertility is attributable to defective production of spermatozoa in roughly 25 per cent of couples. Before embarking on an extensive study of the female partner, evaluation of the male's reproductive potential is mandatory. To an albeit rather limited degree this can be accomplished by semen analysis. The ejaculate is collected into a clean, dry jar and brought to the laboratory as soon after collection as practicable. The age of the specimen is of importance in evaluating sperm motility, and semen analysis should be performed within six hours of collection or sooner. The specimen should not be obtained by means of a rubber condom, as those which are commercially available are treated with spermicidal agents. The patient should be cautioned not to lose the first few drops of the ejaculate, because the majority of active spermatozoa are located in the first portion of the specimen.[6] Although frequent coitus is of little consequence when the ejaculate is normal, the relatively infertile male suffers a marked reduction in sperm production by too frequent ejaculation.[67] The interval from the time of last coitus is therefore of significance, and four to five days of abstinence is usually recommended. The volume, the sperm density, the percentage of motile forms, and the percentage of abnormal forms are routinely recorded.[2, 5, 165]

Standards for Male Fertility

There is no proved relationship between ejaculate volume and ease of conception. There is not as yet complete agreement as to the level of sperm concentration below which a male is to be considered infertile. Some workers feel that the lower level for male infertility should be placed at 60 million per cc, a figure which for years has been accepted as a minimal standard.[50, 1] Recent convincing statistical evidence, however, suggests that above the level of 20 million per cc there is no rise in the potential fertility. It should be kept in mind that pregnancies have occurred when the count has been much lower than 20 million per cc. A low rate of conception has been observed when the motility is under 40 per cent, and there is a high correlation between the quality of motility and the ability to fertilize.[7] Abnormalities in sperm morphology are so intimately bound with the quality of motility that it is difficult to dissociate one from another in evaluating fertility potential.[73] It should be emphasized that once pregnancy has been established, its outcome is in no way related to the quality of the semen.[73, 44]

Clinical Management of Defective Sperm Production

Treatment of deficiencies in sperm production is generally unsatisfactory, as the etiology of the con

dition frequently remains obscure Among a completely studied group of 302 subfertile males Tyler was unable to point to any etiologic factor in 38 4 per cent and an additional 28 4 per cent had hypotrophic testes of obscure etiology [149] A general physical examination with emphasis on the urogenital system is essential The physical cause of decreased sperm production becomes immediately apparent when gross anatomical abnormalities are encountered such as testicular atrophy cryptorchidism or *a significant varicocele A careful history will some*times shed light on the origin of spermatogenic difficulties Mumps with associated orchitis in the post pubescent male may have caused destruction of sperm forming tissues Gonorrhea once a major cause of male infertility has become less of a problem since the availability of antibiotics Ductal obstruction from gonorrhea is still seen however In these cases the testicles are normally formed and their functional status may be established by testicular biopsy Encouraging results with newer techniques for the surgical correction of ductal closure have recently been reported [81 8] Very rarely the males with apparent azoospermia may in fact be having retrograde ejaculation into the bladder This *possibility can be ruled out by examination of the* urine for spermatozoa immediately following orgasm The practical importance of this condition has been emphasized by Hotchkiss *et al* who succeeded in establishing pregnancies by insemination with spermatozoa collected from the bladder [54]

The medical treatment of oligospermia is generally unsatisfactory The use of thyroid extract in euthyroid patients though a popular form of therapy is valueless It may be of some benefit however in those cases with demonstrable hypothyroidism [149] The occasional oligospermic male will respond to suppressing dosages of androgens with an increase in sperm population after cessation of therapy the so called rebound phenomenon [7 151] Response to *androgen therapy is by no means predictable* [43] and adequate criteria for its use have not yet been established Gonadotropins as currently available have no proved value and may even be detrimental [71] A general improvement in constitutional factors such as weight reduction better diet correction of anemia and increased rest are thought to be somewhat beneficial It is most difficult to assess any form of therapy of male infertility because of the known physiologic variations in sperm production from one time to another in the same male [169]

Insemination and the Postcoital Test

During normal intercourse the ejaculate is deposited in the vagina creating a pool in the posterior fornix As ovulation time approaches the cervical mucus undergoes certain physical changes and becomes more receptive to spermatozoa Many of them make their way from the hostile acid environment of the vagina into the life supporting alkaline mucus The spermatozoa remaining in the vagina are killed within one or two hours Of those reaching the cervical mucus *a few progress along the uterine fluid* into the tubes

Though semen analysis is of unquestionable value in assessing male fertility additional information may be gained from the postcoital test The examination is planned to coincide with the estimated ovulatory phase and the patient is instructed to have intercourse the night before her scheduled appointment The test may be routinely performed as long as 18 hours after coitus In fact active spermatozoa have been observed in cervical mucus up to six days following the last exposure [29] After cleansing the cervix of debris a sample of cervical fluid is removed from the os For this a nasal polyp forceps a fenestrated intestinal forceps or a simple aspirator *may be used The quality of the mucus is noted* and the sample is spread on a slide and a coverslip applied

Examination of the cervical mucus postcoitally was advocated by J Marion Sims as early as *1866* [136] In spite of the many years since this first description absolute standards as to what constitutes adequate insemination have not yet been established It is generally accepted however that insemination may be adjudged adequate when at least 10 to 20 spermatozoa are seen in each high power field of mucus The prognostic value of the postcoital test has been questioned by Southam and Buxton who reviewed 70 postcoital tests done in the conception cycle [138] Among these 27 displayed no sperm or only an occasional motile spermatozoan per high power field and yet pregnancy resulted Clift and Hart have described a method for quantitatively evaluating cervical insemination in an effort to standardize the postcoital test [8] Since little is known about the statistics of sperm penetration absolute standards may not be within the realm of possibility Nevertheless a uniform method of performing and *describing the postcoital test* would add to its value

Changes in the Cervical Mucus

Normally there is a cyclic variation in the quality of the cervical mucus throughout the menstrual cycle Immediately following menstruation the mucus is scanty tenacious opaque and cellular Under the *influence of increasing titers* of estrogen there is a gradual change in its quality As the time of ovulation approaches the mucus becomes more abundant thin and clear Its ability to form a continuous thread *(Spinnbarkeit)* increases from 0 1 cm to

10-20 *cm*[30] Its receptivity to spermatozoa is enhanced In the postovulatory phase its characteristics are modified by progesterone and it becomes tenacious opaque and cellular once again These changes in the cervical mucus have been artificially reproduced in castrates by the administration of estrogen and progesterone [11 2 35]

In the evaluation of the cervical mucus its quantity translucence viscosity and microscopic constituents should be noted Its thread forming ability or *Spinnbarkeit* is determined by spreading the blades of the cervical forceps or by lifting the cover slip from the slide until the thread becomes discontinuous (Fig 1) The mucus may be further evaluated by drying it over a flame and examining it microscopically Receptive ovulatory mucus will crystallize into a fern like pattern [1] Such a fern pattern is invariably present in mucus that is grossly clear and fluid and this additional procedure is of no real clinical importance in its evaluation

Management of Defective Insemination

In the occasional infertility patient repeated examinations of the mucus during the ovulatory phase demonstrate that it does not at any time assume the characteristics which would make it receptive When such is the case small doses of estrogen (0 1 0 2 mg of diethylstilbestrol) will sometimes result in the production of more favorable mucus When there is no response to estrogen in small amounts larger quantities may effect a change However a larger dosage of estrogen often inhibits ovulation defeating the primary purpose of the treatment

The quality of the cervical mucus is sometimes influenced by the presence of an endocervical infection There is often gross evidence of an endocervicitis and the mucus remains purulent throughout the cycle In such cases cervical culture and treatment with the appropriate antibiotic have been suggested [4] This initially promising approach has been disappointing as control studies have indicated that there is no difference in pregnancy rate between treated and untreated cases [4]

When the postcoital test discloses inadequate insemination in the presence of what appears to be favorable cervical mucus sperm analysis will sometimes disclose a deficiency When there is oligospermia artificial placement of the husband s sperm with whatever technique is only infrequently successful [9] Earlier experience with the use of a paracervical insemination cup was encouraging [1 9] Some success has been reported with husband insemination when the ejaculate is reasonably good but postcoital tests show consistently poor insemination [9] The presence of anatomical abnormalities which would interfere with proper natural placement of semen should be looked for Mastroianni and Rock have described a group of infertile patients in whom there was anatomical evidence that intromission during intercourse had been incomplete at best and perhaps had never been achieved as indicated by an

CERVICAL MUCUS CHANGES IN THE MENSTRUAL CYCLE

FERTILE PERIOD	INFERTILE PERIOD
1 THIN PROFUSE CLEAR	THICK SCANT, TURBID
2 EASY TO ASPIRATE	DIFFICULT TO ASPIRATE
3 SPINNBARKEIT (THREAD FORMATION) 10 TO 20 CM	SPINNBARKEIT (THREAD FORMATION) 0 TO 1 CM
4 SPERM SURVIVAL OPTIMAL GOOD MOTILITY NUMEROUS SPERMATOZOA	SPERM SURVIVAL POOR POOR MOTILITY 0 TO FEW SPERMATOZOA
5 CELLS NORMALLY ABSENT	CELLS USUALLY PRESENT

Fig 1 The qualities of cervical mucus which are routinely recorded with the postcoital test (Courtesy of Cohen M Stein I F., Sr and Kaye B M Fert & Steril 3 201-8 1953)

intact hymen or very rigid perineal body [9] The use of graduated vaginal dilators with or without initial perineorrhaphy and frequent consultation usually resulted in the institution of normal intercourse and pregnancy frequently followed

In every case of inadequate insemination examination of the male is necessary regardless of whether sperm analysis is within the normal range Interview with the husband may disclose sexual difficulties such as inability to maintain erection or premature ejaculation which the female may find difficult to discuss *Occasionally the examiner is rewarded by* the disclosure of the presence of hypospadias This condition might result in loss of much of the ejaculate and insemination with the husband s sperm is the treatment of choice Not infrequently there is a history of rising from bed following intercourse or even postcoital douching for cleanliness Advice on such matters often results in sufficiently improved insemination to establish a pregnancy

TUBAL FACTORS IN INFERTILITY

It has been estimated that in as many as 15 per cent of infertile marriages abnormalities in the fallopian tubes can be directly implicated as causative [115] That the fallopian tubes are not merely passive transporting passages is evident clinically from the numbers of cases with pathological tubes where although patency has been re established surgically and maintained pregnancy fails to result

Physiological Considerations

The tubes are clearly dynamic organs It has been demonstrated that there are cyclical variations in the morphology of the human endosalpinx [87] Recently Balboni has reviewed the earlier work on this subject and has used histochemical techniques to clarify *the details of these changes* [5] *He has described several* cellular types in tubular epithelium ciliated cells very numerous in the follicular phase and poor in ribonucleic acid transitional cells rich in cytoplasmic nucleolar ribonucleic acid which lose their cilia and become secretory secretory cells more numerous premenstrually clear cells poor in nucleotides which become numerous near the end of the cycle and are considered to be exhaustion cells nucleo protein synthesizing cells present early in the cycle and supporting peg or stick cells Balboni has also demonstrated cyclical variations in the ground substance of the tubal stroma [6] In the proliferative phase the ground substance is homogenous and strongly PAS reactive The basement membrane is thick In the postovulatory phase the ground substance appears thinner and scleroreticular and the basement membrane becomes thinner It is tempting to conjecture that these morphological changes are in some way related to tubal function in reproduction

Cyclical variation in the contractility of the human fallopian tube *in vitro* has been described by Seckinger and Snyder [1] They noted an increase in both rate and amplitude of contractions at about mid cycle Manzi demonstrated that tubal contractions become deeper and more regular under the influence of follicular fluid and that extract of corpus luteum almost stopped tubal motion [77] A variation in the metabolism of the endosalpinx in the menstrual cycle as determined by oxygen uptake studies with the Warburg apparatus has been shown by Kneer *et al* [63]

About 14 days preceding the menstrual period the ovum having undergone a maturation division is released along with follicular fluid from the mature follicle [107] It must traverse the distance between the ovary and the tube Normally the ovary is held *in reasonable proximity to the tubal ostium* by the utero ovarian ligament Westman who has actually witnessed ovulation through an abdominal window in the rabbit and by means of a pelviscope in the monkey observed that the smooth muscle fibers of the adnexal ligaments bring the ovary closer to the tube during ovulation [156] The fimbria of the tube actually embrace the ovary [157] However the rare cases of pregnancy where there is but one ovary and but one tube on the contralateral side promote speculation that proximity of the tube and ovary may not always be essential There are currents within the tubal lumen capable of transporting foreign material from the cul de sac into the uterus [1 4] It is therefore not inconceivable that an ovum deposited in the cul de sac might be picked up by the tube

The ciliary action of the human tube has been studied by Kneer and Cless [64] They describe a brisk *forward movement of the cilia toward the uterus* and a slower backward motion Tubal fluid is thereby moved in waves They postulate that in the wider portions of the tube backward motion of the ovum toward the fimbria is prevented by folds of the mucosa Thus the ovum is not caught *in the back wash* as it were and continues its slow progression toward the uterus Spermatozoa on the other hand are propelled by their flagella They distribute themselves in the periphery of the stream and are prevented from flowing retrograde by the salpingeal folds In the narrow isthmic portion of the tube peristalsis may play a role in ovum transport

In addition to its function of transportation there is evidence that the tubal mucosa is important in the process of fertilization Shettles has demonstrated that tubal mucosa is capable of dissolving the corona and cumulus cells surrounding the human

ovum[129] He has added crushed tubal mucosa to his culture medium to obtain a morula stage fertilized human ovum *in vitro*[130] It is evident that the function of the fallopian tube is complex indeed and is worthy of continued investigation

Tubal Pathology Resulting in Sterility

Inflammatory conditions of the fallopian tubes may result in damage to the delicate endosalpinx and partial or complete obliteration of the tubal lumen Before the age of antibiotics the gonococcus was the prime offender and tubal damage as a result of gonorrheal salpingitis ranged in incidence from 3 per cent in Siegler's collected series of private cases to 70 per cent in the Far East[134] Gonorrheal salpingitis affects the endosalpinx first gaining entry to the tube along the surfaces of the mucous membrane Usually there is conglutination of the fimbria resulting in varying degrees of closure When occlusion occurs at the isthmus as well as the fimbriated extremity there is accumulation of exudate within the tube with dilatation subsequent clearing of the exudate and the formation of a hydrosalpinx

Salpingitis also occurs as a result of postabortal or postpartum infections In every case of secondary infertility hospital records of previous pregnancies should be examined and any signs of puerperal infection noted Infection from puerperal sepsis reaches the tubes by vascular and lymphatic spread In the acute phase the tubal wall the mesosalpinx and the broad ligament are involved and the endosalpinx is relatively spared Peritubal adhesions often result Patency is frequently maintained but when closure does occur it is more common at the isthmus

In recent years pyogenic infections of the tubes have played a less prominent role in infertility and attention has been focused on tuberculous salpingitis The incidence of this condition varies geographically being relatively more common in the underdeveloped areas of the world Sharman reports the incidence of genital tuberculosis among a large group of infertility patients in England as 5 2 per cent A history of pulmonary tuberculosis should alert the physician as to the possibility of tuberculosis in the pelvis The finding of a negative chest roentgenogram does not however rule out genital tuberculosis pulmonary lesions being demonstrable in only about one third of patients Tubercle bacilli usually reach the tubes via the hematogenous route from a distant source commonly the lungs The organism may also spread by continuity or rarely by way of lymphatics[3] The macroscopic appearance of the tuberculous tube is not distinctive except when it is involved secondary to tuberculous peritonitis and is studded with tubercles When there is a tuberculous pyosalpinx a caseous exudate is seen on cross section As with gonococcal salpingitis tuberculous salpingitis is invariably bilateral The fallopian tubes are involved in 90 100 per cent of cases of genital tuberculosis The endometrium is involved in about 50 per cent and unsuspected pelvic tuberculosis is sometimes detected by endometrial biopsy or curettage Sharman found the tubes to be closed to insufflation in 67 per cent of cases with genital tuberculosis[126]

Tubes may be involved secondarily as a result of pathology having its origin elsewhere Appendicitis diverticulitis and generalized peritonitis may cause pelvic and peritubal adhesions A suggestive history should be thoroughly investigated A ruptured appendix is more likely to be of importance if the episode occurred in childhood when the appendix and pelvic organs are in close proximity Pelvic endometriosis and previous pelvic surgery may also be responsible for pelvic adhesions involving the fallopian tubes

Clinical Evaluation of the Fallopian Tubes

In 1920 Rubin described the technique of uterotubal insufflation[11] This simple office procedure has become a mainstay in the armamentarium of those concerned with the study of human infertility The test is preferably performed in the preovulatory phase of the cycle The apparatus used consists of a curved cannula with a rubber adapter designed to fit snugly at the cervix placed 5 6 cm from the end Any one of several commercially available insufflating machines is suitable for the introduction of carbon dioxide Some of these are designed to register the rate of flow of gas as well as the pressure at which it is being introduced A few are equipped with a kymograph to give a tracing of the pressures used A preliminary pelvic examination should always be performed to determine the position of the uterus as well as to detect the presence of adnexal disease The test should be avoided if any hint of an active inflammatory process is present The cervix is exposed with a bivalve speculum and cleansed Carbon dioxide is allowed to escape through the cannula to insure its patency and the instrument is introduced into the uterine cavity so that the rubber adapter is flush with the external cervical os When there is an element of cervical stenosis or a very patulous cervix it may be necessary to create counter pressure with a vulsellum to insure an air tight communication Carbon dioxide is allowed to pass into the uterus at a rate of 30 to 60 cc per minute The pressure at which the gas is introduced should not be allowed to exceed 200 ml of mercury Rubin has found that auscultation of the abdomen during insufflation gives useful information an intermittent bruit being produced as the gas escapes from the tubes

Following insufflation the patient is asked to sit upright If gas has escaped from the tubes into the abdominal cavity it rises irritating the diaphragm and causing referred shoulder pain mediated via the phrenic nerve Tubal patency cannot be considered to have been established as fact unless there is shoulder pain Gas may fill a dilated closed tube at a constant pressure and rate causing gradual distension of the organ without escape into the peritoneum In such a situation there is no shoulder pain although there is apparent passage of the gas at a low pressure Shoulder pain cannot of course be elicited if the procedure is performed under anesthesia

Rubin has stressed the diagnostic value of kymographic tracings during insufflation The oscillations registered are thought by him to represent tubal peristalsis and are felt to be valuable in differentiating various types of tubal pathology [113, 116, 7] Stabile however has recently presented evidence that the oscillations in pressure are of myometrial origin being produced by a rhythmic sphinctoric action of the myometrium upon the intramural portion of the tube [140]

Tubal insufflation has been considered by many to be of therapeutic value in infertility [11, 25] It is certainly true that pregnancy does sometimes follow insufflation but it is difficult to assess the role of the procedure per se in these cases Sharman obtained pregnancies in 17 per cent of patients by the introduction of a uterine sound alone [126] Data ascribing therapeutic value to any procedure in infertility should be interpreted with this in mind

Uterine and tubal pathology may be uncovered by hysterosalpingography This is a more elaborate procedure than insufflation as roentgenological equipment is required Hysterosalpingography is clearly indicated whenever there is any doubt about tubal patency as demonstrated by insufflation Further the procedure should be performed even in the face of an apparently satisfactory insufflation if pregnancy does not ensue within a reasonable time Greenhill has aptly pointed out that hysterosalpingography and insufflation should not be looked upon as competing methods of diagnosis and that each has its special field of usefulness [49] Hysterosalpingography should be performed at a time in the cycle before ovulation to obviate the possibility of the presence of an early pregnancy Radiopaque material is introduced into the uterus and tubes by means of a cannula similar to that employed in insufflation It is important to be sure that the cannula has been filled with media before starting the procedure Failure to do this will result in introduction of air bubbles which may create artifacts Films are taken at appropriate intervals during the injection of the dye Fluoroscopy is thought by some to facilitate diagnosis [1, 6, 9] Seldom is more than 4 cc of radiopaque material required to outline the fallopian tubes and establish the presence of patency [151] This fact is particularly significant when an oily medium is used Larger quantities of oily material are retained in the peritoneal cavity for long periods of time and foreign body reaction to the material is known to occur [16] When there is nonpatency or peritubal adhesions the dye is retained in and about the tube absorption is delayed and granulomatous reaction becomes a distinct possibility [118] In addition the occasional intravasation of the medium raises the possibility of oil embolism [7, 13] For these reasons opposition to the use of oily media has been voiced [98, 118] Those favoring the use of oil point out the diagnostic advantage of the delayed follow up film [17, 34] Pelvic adhesions may be identified on x ray several hours after introduction of the dye because they localize the distribution of the dye forming a distinctive pattern

As with insufflation there are those who feel that hysterosalpingography is of therapeutic value in infertility [111, 135, 153, 168] Data presented by Buxton and Southam have cast doubt on any therapeutic effectiveness [2] Their importance as diagnostic aids it should be stressed is beyond question

In 1944 Albert Decker described his technique for telescopic visualization of the female generative tract (Fig 2) [37] His method differed from earlier approaches in that he employed the knee chest position for the procedure Adequate visualization of the tubes the ovaries and the posterior and lateral aspects of the uterus is usually afforded Because culdoscopy permits assessment of anatomical abnormalities without resorting to laparotomy it has become a valuable diagnostic tool in infertility studies It is used to advantage to confirm the presence of pelvic adhesions which are suspected either from the history or the hysterosalpingogram Asymptomatic adhesions undetected by insufflation or hysterosalpingography are diagnosable by culdoscopy and explain previously inexplicable infertility [00, 03, 4] Kelly and Rock reported that among 417 successfully performed culdoscopies for infertility in 84 unsuspected pelvic pathology was uncovered [61] An intelligent decision for or against laparotomy was thereby permitted

Positioning of the patient is an important step in the technique of culdoscopy Decker advocates the use of a footboard and shoulder braces for support The vagina and perineum are prepared with an antiseptic solution The cervix is exposed with a right angle retractor and its posterior lip secured with a curved vulsellum If the cul de sac is free the posterior fornix will balloon toward it When a local anesthetic is employed superficial wheals are created in the most concave portion of the posterior fornix Using the vulsellum for counter trac

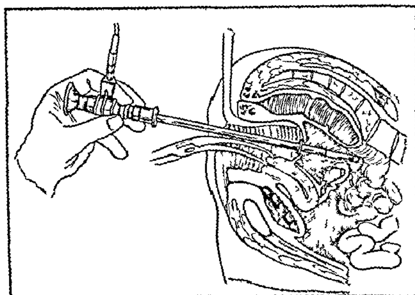


FIG 2 APPRAISAL OF OVARIES AND TUBES BY CULDOSCOPIC EXAMINATION (Courtesy of *Modern Medicine* 1956)

tion the trocar and cannula are introduced with a deliberate thrust If the puncture is successful the sound of inrushing air will be heard on removal of the trochar from the cannula and there will be no resistance on introducing the culdoscope For a detailed treatment of the technique of culdoscopy the reader is referred to the excellent monograph by Decker[36]

Culdoscopy is not possible when there is a fixed retroversion This condition is in itself justification for laparotomy when it is associated with infertility of long duration all other factors being normal It should be stressed however that the failure to conceive is probably not attributable to the retroversion per se but to the associated pathology which causes the retroversion to be fixed

Treatment of Anatomical Abnormalities of the Tubes

Claims for therapeutic procedures short of surgery for anatomical derangements involving the fallopian tubes remain open to continued scrutiny Surgical treatment is still far from completely satisfactory When there are pelvic adhesions which might conceivably interfere with ovum transport laparotomy for lysis is justifiable provided other factors which might be contributing to infertility have been eliminated Certainly no surgical procedure on the tubes is warranted in a patient who is not ovulating or in whom insemination is inadequate When there is occlusion at the fimbriated extremity Mulligan and Rock recommend that a polyethylene hood be placed over the reconstructed ostium to prevent the reformation of adhesions about the fimbria[85 109] The hood is removed at a second laparotomy in six to eight weeks (Fig 3)

When the tube is occluded in its proximal portion the use of polyethylene tubing has been suggested to maintain patency during epithelialization following cornual reimplantation[8 10] The tubing is threaded through the uterus anchored to the cervix and is left *in situ* for at least six weeks In Mulligan and Rock s series tubal patency was maintained in 57 per cent of fimbrioplasties and 44 per cent of tubal implantations Pregnancy resulted in 33 per cent and 9 7 per cent respectively[1 8] These encouraging results represent marked improvement over the earlier ones which prompted Greenhill s statement in 1937 based on statistics gathered from leading centers that tuboplasty was not a justifiable procedure[*] It should be stressed that tuboplasties are delicate surgical procedures Fine dissecting instruments probes and other special equipment are required Ideally the operation should not be attempted except by those thoroughly familiar with the specialized techniques involved

DIAGNOSIS OF OVULATION

In every infertility problem it is of fundamental importance to determine the presence or absence of ovulation The hormonal changes resulting in the ovulatory process have been considered in detail in the chapter by Garcia and Rock Suffice it to say here that secretion of the hormone progesterone is instituted at or about ovulation time The most practicable of the presently available tests for the detection of ovulation depends on the influence of this hormone on the reproductive system

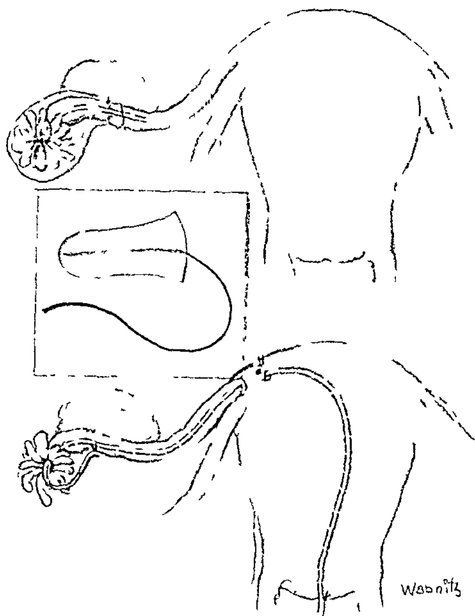


Fig 3 Techniques of tuboplasty using polyethylene Insert (left center) shows molded polyethylene hood Above hood is pictured in place following fimbrioplasty after the method of Mulligan and Rock [3, 10, 9] Hood having prevented fimbrial adhesions is removed 6-8 weeks later Below polyethylene tubing is shown in place following tubal implantation for cornual obstruction Removal is accomplished from below after a suitable interval

An impression as to whether ovulation is occurring may be gleaned from the patient's history When there are regularly occurring menstrual periods which are of equal duration it is fairly safe to assume that the cycles are ovulatory [10, 147] Normally menstruation follows ovulation within 12 to 16 days [107] Not uncommonly ovulation is accompanied by abdominal discomfort the so called *Mittelschmerz* Staining or frank bleeding accompanies ovulation in 9 per cent of normally menstruating females [91] Bleeding is detectable microscopically during the ovulatory phase in 60 per cent of women [1, 3] and occult bleeding as demonstrated by the benzadine test in 90 per cent [14] *Mittelschmerz* and bleeding frequently pass unnoticed until attention is focused on them by the physician

The temperature chart is of established value in the diagnosis of ovulation (Fig 4) Progesterone being thermogenic causes a rise in the basal (waking) temperature in the postovulatory phase of the

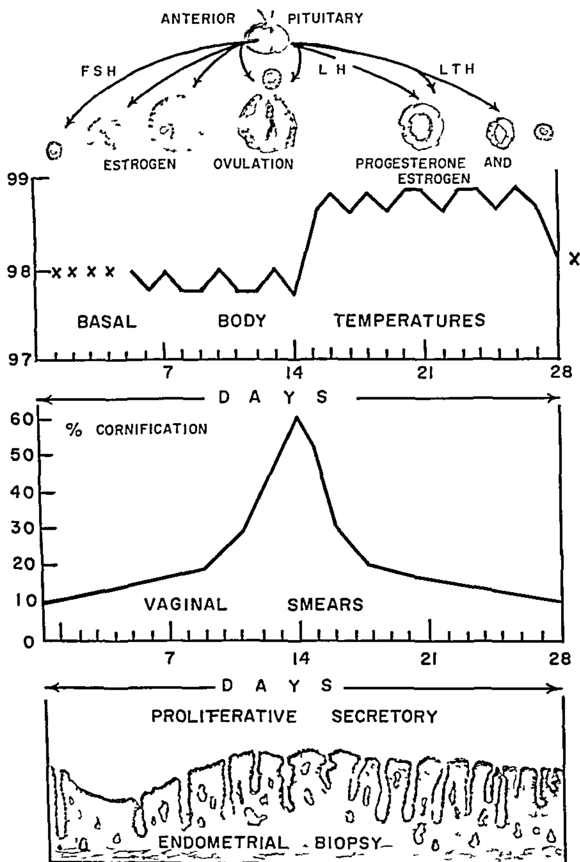


Fig 4 Illustration of the various methods which are useful in the clinical diagnosis of ovulation

cycle [4] The patient is instructed to record her morning temperature before rising from bed Oral temperatures are as satisfactory as rectal ones Typically within 48 hours of actual ovulation there is a drop in basal temperature which is followed by a sustained rise [9] There is usually a fall in temperature again just preceding menstruation If pregnancy has ensued the temperature is maintained at the post ovulatory level presumably by continued activity of the corpus luteum [35,148] No clinical significance can be ascribed to the quality of the temperature rise which although usually abrupt may be gradual over two to three days Temperature charts cannot effectively be used to determine ovulation time until after the fact and are useful in the timing of ovulation only in a general way It is important that this be emphasized to the patient as efforts to time intercourse according to changes in the temperature chart often result in tension and frustration

Release of progesterone causes modifications in endometrial morphology which are progressive and predictable [3,9] These have been described in detail in the chapter on menstruation A sampling of the endometrium for study is obtained by biopsy Any of several specially designed endometrial biopsy curettes is suitable After determining the position of the uterus by bimanual examination the cervix is exposed and cleansed Its anterior lip is grasped with a vulsellum and the curette is guided beyond the internal os into the uterine fundus It is then withdrawn coursing along the anterior or posterior uterine wall The tissue obtained is placed in a suitable *fixative such as Bouin s solution* This procedure may be performed in the office without anesthesia and results in only minor discomfort The danger of interrupting a gestation is obviated if the biopsy is done before the fifth or sixth postovulatory day During this interval the ovum is still either in the tube or in the uterine fluid Implantation is believed to occur from the fifth to the eighth post ovulatory day [106] Beyond this time the possibility of damaging an implanted ovum is increased At that the risk is small as its size at the time of the expected period is only 1 5 mm [3] Some gynecologists prefer to perform the biopsy on the first day of menstruation This would practically insure avoiding the interruption of a pregnancy and would give a better indication of endometrial development The appointment cannot be scheduled however and menstruation may occur at a time inconvenient for both patient and physician In the dating of ovulation there is good correlation between temperature chart and endometrial biopsy [5] As is the temperature chart endometrial biopsy is useful in timing ovulation only after the fact but *for this it is invaluable*

Serial vaginal smears may be used for ovulation detection The staining methods of Shorr are the most satisfactory for this purpose [131,3] Interpretation of the smears is based mainly on the changes brought about by estrogen *The estrogen level reaches a peak* immediately preovulatorily causing maximal cornification of the cells in the desquamate [9] Other cytological criteria are important such as clearing of the desquamate the appearance of mucus and the sudden arrival on the scene of leukocytes preovulatorily [45] For proper evaluation several smears taken in successive days in the *pre- and postovulatory* phases are required A single smear is of no value for the diagnosis of ovulation It is possible to teach the patient to take the smears herself on numbered slides and store them in fixative until her next office visit Vaginal smears are not as practical as the less complicated basal body temperature and biopsy as they require either patient training or frequent office visits as well as greater experience in their interpretation

The rat ovarian hyperemia test as described by Farris has been used for the diagnosis and timing of ovulation [31,40,94] The method is based on the observation that during the four days preceding ovulation the urine contains a gonadotropic substance which produces hyperemia of the ovaries of immature rats Urine is administered subcutaneously and the ovaries are removed and examined colorimetrically after two hours Normally hyperemia is said to be produced for four consecutive days ovulation occurring on the last day of the reaction The accuracy of the test has been challenged [9] As it would require daily collections of urine and the maintenance of a rat colony it is of questionable practicability for the average clinician

The various methods of ovulation detection are but measurements of associated hormonal changes The only circumstances under which the physician may be absolutely assured that ovulation has occurred are when pregnancy is subsequently established or when an ovum has been recovered from the fallopian tube The intriguing possibility has been raised that conceivably the egg may mature and a corpus luteum be formed without actual escape of the ovum from the ovary [10] It is well known that this can occur in other species The mechanism by which *ovulation is finally triggered is as yet* unknown Ovulatory failure can however be diagnosed with certainty when there is no demonstrable progesterone effect during the cycle The cause for the anovulatory state should be sought for by a carefully planned diagnostic program The difficulty may originate in the hypothalamus the pituitary or the ovary *per se* or it may result from extra-ovarian extra pituitary hormonal influences

EVALUATION AND TREATMENT OF OVULATORY FAILURE

It is known that there is a nervous pathway from the hypothalamus to the pituitary gland In addition recent work hints of a hormonal link between the two organs[5] The influence of the psyche on ovulation has been well documented [6 98] It would seem reasonable that the hypothalamus is the medium through which this influence is channeled The intricacies of the mechanisms involved remain obscure and as with many of the more complicated problems in psychiatry diagnosis is made by exclusion Cases of so called hypothalamic amenorrhea often spontaneously revert to normal menstrual activity after simple reassurance For patients with more deep seated psychological disturbances intensive psychotherapy may be required

Influence of the Pituitary Gland

Hypopituitarism is rare among patients complaining of infertility It is usually characterized pathologically by atrophy and fibrosis of the anterior lobe secondary to acute necrosis and is seen in patients who have suffered a severe obstetrical hemorrhage[127] It may also occur secondary to pituitary tumor The activity of the adrenals thyroid and ovaries is secondarily involved Gonadal failure is usually the first to occur however and amenorrhea may be the only evidence of the illness for many years[39] Signs of hypoestrogenism to be discussed below are present The hypoestrogenism secondary to hypopituitarism can be differentiated from ovarian hypoestrogenism by urinary FSH levels FSH production is low or absent in the former condition In the latter FSH levels are high the normally functioning pituitary having been released from the braking action of estrogen When pituitary damage is not severe ovulation and therefore menstruation may continue and pregnancy may rarely occur When there is amenorrhea the prognosis for childbearing is poor There is no known treatment as suitable hormonal replacement therapy is not yet available

Ovarian Disturbances

Patients with primary ovarian failure may be conveniently divided into two broad categories Those in which there is a complete arrest of ovarian function and those in which estrogen production has continued but ovulation has ceased Included in the former group are patients with early cessation of ovarian function the so called premature menopause as well as the unusual cases of ovarian agenesis They present the following evidence of estrogen deficiency endometrial biopsy reveals an inactive endometrium repeated vaginal smears show absence of cornification of the cells withdrawal bleeding does not follow the administration of exogenous progesterone because the endometrial bed has not been prepared by estrogen and urinary FSH levels are elevated The cause of premature failure of estrogen production is unknown and treatment of infertility resulting from such a disturbance is unsatisfactory The patient may present symptoms of vasomotor instability characteristic of the menopause Symptomatic relief is afforded by estrogen treatment which when administered cyclically in adequate dosage will often cause periodic bleeding In patients with ovarian agenesis estrogen production has been impaired from the start and the secondary sex characteristics fail to develop Ovarian tissue is present in minimal amounts or absent completely The uterus when present at all is tiny and the vagina poorly developed Typically the patient is short of stature and the breasts are widely spaced and small When associated with infantilism webbed neck and cubitus valgus such cases have been designated as Turner s syndrome[4] Study of the chromosomal pattern with buccal smears and skin biopsies has disclosed that many cases of ovarian agenesis are in fact chromosomally male[63] There is no known treatment except for replacement therapy with estrogens to bring about development of secondary sex characteristics Such patients may be told that their infertility is irrevocable and advised to plan their lives accordingly

In the majority of infertility patients with ovarian dysfunction estrogen production continues but there is failure of ovulation (Fig 5) The result is amenorrhea or more often a menstrual pattern that is irregular in both time and duration of flow This dysfunctional flow occurs when the endometrium is built up to a point beyond which the estrogen level is unable to support continued growth Bleeding occurs from a proliferative type of endometrium The temperature chart fails to exhibit an ovulatory rise and endometrial biopsy at or near the time of menstruation reveals tissue showing evidence of estrogen stimulation only This variety of menstrual behavior is more frequently seen among girls just beyond the menarche or in patients approaching the menopause Bleeding is sometimes prolonged and heavy and secondary anemia may result Hormonal therapy is invaluable in controlling irregular and distressingly prolonged periods A periodic injection of 25 50 mg of crystalline progesterone will cause withdrawal flow at controlled intervals[31] Such treatment prevents excessive build up of the endometrial bed limiting the amount of flow with each induced period Holmstrom has reported the re

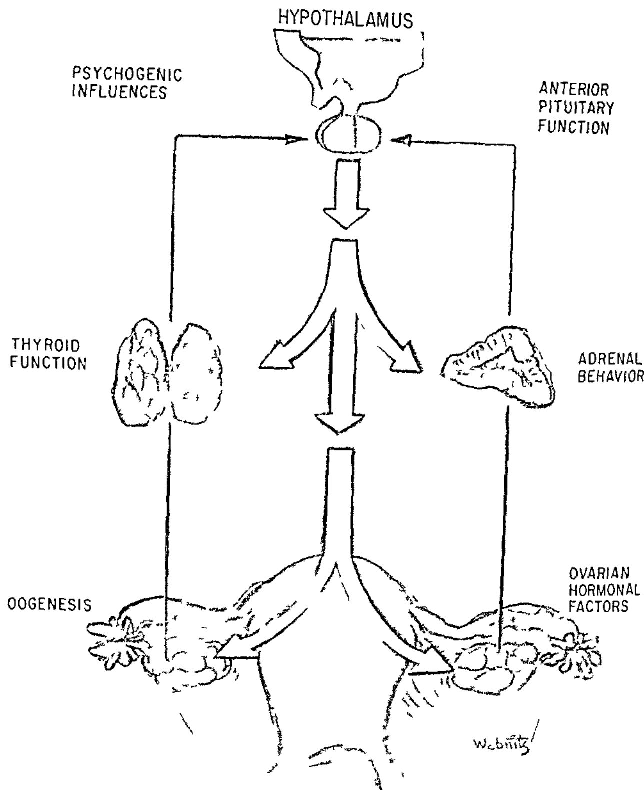


FIG 5 ILLUSTRATION OF THE ENDOCRINOLOGICAL PATHWAYS THROUGH WHICH OVULATION MAY BE STIMULATED OR INHIBITED

establishment of ovulation during cyclic progesterone therapy in many of his cases so treated [1] Swartz and Jones however have been unable to confirm this among a small group of cases [143] Judgment must be withheld until additional data are forthcoming

Claims for the hormonal induction of ovulation are difficult to evaluate at best Buxton and Southam have carefully analyzed data from a large group of anovulatory patients and report that the pregnancy rate did not differ appreciably between those

who were treated hormonally by various methods and a similar group of untreated cases [23]

Polycystic Ovaries

Since the report of Stein and Leventhal in 1935 [14] attention has been focused on the polycystic ovary as an etiological factor in cases of ovulatory failure These workers described the association of amenorrhea hirsutism and bilateral polycystic ovaries and the syndrome bears their names (Fig 6) The *ovaries are frequently enlarged* and the capsule is thickened tense and glistening with multiple small cystic follicles beneath it Although the majority of patients presenting such an ovarian picture are amenorrheic a few present a history of menometrorrhagia *Three fourths of cases have a somewhat* hypoplastic uterus and the breasts are underdeveloped in somewhat less than half [41] Stein has recommended pneumoroentgenography in combination with hysterosalpingography as a useful method of diagnosis [141] By this means roentgenologic visualization of the bilaterally enlarged ovaries is possible Actual visualization of the ovaries may be accomplished with culdoscopy their typical appearance being unmistakable The etiology of polycystic ovaries is still a matter for conjecture Stein has stated The development of this condition is not congenital neither is it inflammatory nor degenerative It is the result of a definite endocrine disturbance [1] Ingersoll and McDermott have reported that FSH assays are within normal limits They have postulated a deficiency in the production of luteinizing hormone by the anterior pituitary as the fundamental deficiency [55] Recently the possible relationship between adrenal function and the Stein Leventhal syndrome has been probed [5] but the mechanism by which adrenal influence is mediated is not yet clear Treatment with cortisone in those cases exhibiting *in addition to polycystic ovaries* evidence of hyperadrenalism in the form of elevated 17 keto steroid excretion has yielded promising results [5 5] With this exception these cases have proved refractory to hormonal therapy It is encouraging however *that in this group of patients the physician can* offer definitive therapy in the form of bilateral wedge resection of the ovaries Such a procedure affords a good prognosis provided the cases are properly selected The operative technique as originally described by Stein and Leventhal involves the simple removal of a wedge of ovarian tissue with light resuturing to secure hemostasis Modifications of this technique have been suggested with no improvement in end results [4 93] In his series Stein reports restoration of menstrual function in 95 per cent and pregnancy in 88 7 per cent [141] These cases are not com

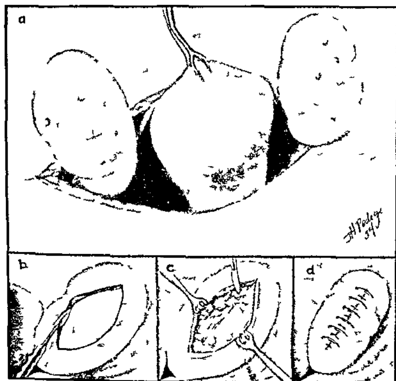


FIG 6 ILLUSTRATION OF POLYCYSTIC OVARIES OF THE STEIN LEVENTHAL VARIETY AND THE TECHNIQUE OF OVARIAN WEDGE RESECTION USED FOR THEIR CORRECTION (Courtesy of I F Stein Sr Fert & Steril 6 190 202 1955)

mon Stein having selected only 88 patients for wedge resection in the past 25 years[141] Recognition of the condition among infertility patients is especially *important because therapy that is of established* value can be offered The reason for the success of wedge resection in the treatment of polycystic ovaries is as obscure as its etiology

Adrenal Influences

There is unquestionably an interrelationship between adrenal cortical activity and gonadal function In the extreme cases with congenital adrenal hyperplasia the resulting clinical picture is that of female pseudohermaphroditism The detailed features of this condition have been reviewed elsewhere in this volume Among these is a suppression of ovarian activity with consequent amenorrhea When cortisone is administered feminization results and in those patients in the adolescent age group and beyond the onset of ovulatory menstrual periods has been observed[6,164]

When adrenal hyperplasia has its onset late in childhood or in adult life its manifestations are less extreme Typically there is excessive growth of hair on the face limbs and trunk acneiform facies and obesity Urinary 17 ketosteroid excretion is elevated As in patients with congenital adrenal hyperplasia ovarian function is impaired and ovulation may be instituted by suppressing the adrenal cortical hyperactivity with cortisone[4,67,161] A plausible explanation for the therapeutic effect of cortisone on ovarian function in these cases has been advanced by Wilkins *et al*[161] The production of androgens and estro*gens by the hyperactive adrenal is greater than normal* These inhibit the production of gonadotropins by the anterior pituitary Decreased ovarian activity results When cortisone is administered the production of ACTH by the pituitary is inhibited Stimulation of the adrenal cortex is decreased and its activity is diminished The production of adrenal androgens and estrogens is lessened and they are no longer present in amount sufficient to inhibit output of pituitary gonadotropins The brake on gonadotropin production having been released ovarian stimulation is once more accomplished

Fifty mg a day of cortisone acetate or its equivalent is usually sufficient to suppress adrenal hyperactivity in these cases The contraindications to the use of cortisone should be carefully observed It should be withheld in patients with a history of mental illness or pulmonary tuberculosis Ideally tuberculosis should be ruled out by a preliminary chest roentgenogram During treatment the weight blood pressure urine and mental attitude should be evaluated at frequent intervals The effect of cortisone on adrenal function can be evaluated by 17 ketosteroid determinations during treatment

Several cases have been reported in which cortisone was thought to be effective in establishing ovulation in the presence of normal 17 ketosteroid values[47,67] Its use has even been extended to patients with ovulatory cycles and inexplicable infertility[41] Final judgment as to the actual value of the drug in such situations must await further clinical trial

The Thyroid and Ovulation

The thyroid gland has long been implicated as causative in patients with ovulatory disturbances It is generally agreed that when there are extreme degrees of deviation in thyroid activity there are usually accompanying disturbances in ovarian behavior[46,60] These express themselves by abnormal menstrual behavior which is generally characterized by amenorrhea and oligomenorrhea in thyrotoxicosis and dysfunctional uterine bleeding in myxedema[10,46] Appropriate therapy invariably results in return to a normal menstrual pattern Subclinical deviations in thyroid activity are also thought to influence ovarian function and treatment when limited to those cases in which the diagnosis has been substantiated by adequate laboratory studies is frequently rewarding[21,149] There is no doubt that thyroid medication has been used indiscriminately for treatment of ovarian disorders associated with infertility and even empirically in cases of infertility with normal ovarian function That it is not of any value in such cases has been repeatedly demonstrated[21,3,149] The evaluation of thyroid function by BMR alone is fraught with inaccuracies Blood iodine determinations give a more adequate appraisal of thyroid function[120]

Radiation Therapy for Ovulatory Disturbances

Radiation therapy to the ovaries for the treatment of ovarian dysfunction has been given considerable attention in the literature Therapy has been extended to the hypophysis as well as the ovary though the value of pituitary irradiation has been questioned[105] It is difficult to evaluate this method of treatment because in the majority of reports there has been little attempt to classify the cases by diagnostic endocrinological studies The impression gathered is that they fall into the broad category of patients with ovulatory failure and continued estrogen production The recommended total dose delivered to the ovaries has ranged from 100 r[6,10] to as high as 225 r[69,94] which is divided into three or four weekly treatments Israel has stressed that ovarian irradiation is contraindicated in patients

with amenorrhea and but one ovary in girls under the age of 20 and when the possibility of an early pregnancy has not been excluded[56] It is to be avoided also in patients over 35 years of age[3 105] The results of x ray therapy are worthy of considera tion Among 90 amenorrheic women in Israel s series 24 conceived within six months of treatment Equally encouraging experience has been reported by others[31 90] Yet ovarian irradiation is remarkable for the divergence of opinion as to its justifiability Objections have been raised by geneticists and gynecologists alike who fear the production of mu tations Muller[9] Russell[121] and Rugh[119] have pointed out the possible harmful effect of mutations on future generations Rubin[11] and Kaplan[60] coun ter with a third generation follow up population of *normal offspring and point out* that no acquired lethal effects have been noted in hundreds of in fants born after ovarian irradiation The clinician treating infertility must weigh the evidence pro and con and decide as to its justifiability Certainly treat ment should never be instituted until ovulatory fail ure has been definitely diagnosed and other thera peutic measures have failed

Luteal Phase Disturbances

Occasionally the infertility patient presents clinical evidence of ovulation which is followed by a luteal phase that may be considered to be deficient The temperature chart reveals a postovulatory rise that is sustained for less than 12 days and biopsy at menstruation shows a poorly developed secretory endometrium[8] This atypical secretory phase has been ascribed to premature involution of the corpus luteum[166] Patients presenting the picture have been known to be capable of conception without therapy and the relationship between it and the sterile state remains uncertain Small doses of estrogen pro gesterone in the postovulatory phase[4] and chorionic gonadotropin[1] have been recommended The effec tiveness of these various forms of therapy has not as yet been established

It is evident that ovarian physiology is complicated indeed and still only poorly understood The known causes of ovulatory failure are multiple and treat ment is varied Only by careful use of presently available diagnostic measures can the clinician be most effective in this management of disorders of ovulation and their associated infertility

THE EMOTIONAL FACTORS IN INFERTILITY

Patients seeking help for infertility frequently con ceive during the diagnostic work up Not uncom monly conception ensues after nothing more than an examination with the *promise of a diagnostic* program to follow[2] It is tempting to conjecture that in such cases psychological factors were operative in preventing pregnancy and that somehow the re assuring demeanor of the physician brought about the necessary mental changes

Kroger and Freed have recommended that the diagnosis of psychogenic sterility be based on failure to conceive after complete study of both partners has uncovered no abnormalities Such patients have been described as poorly adjusted psychosexually *and to have inferiority feelings a desire to remain* childlike and a grasping for affection and sympa thy[167] The somatic means through which psycho logical conflicts are expressed in this situation re mains obscure In fact save for the cases of hypo thalamic amenorrhea and those where coitus has *been infrequent for emotional reasons no physio* logical mechanism has been demonstrated to explain the somatic effect of the psyche on reproduction Tubal spasm has long been suspected Assuredly spasm is often observed in tense anxious patients during insufflation and hysterosalpingography There is no evidence however that the spasm can be sus tained for periods of time long enough to interfere with the reproductive processes

Certainly there is an emotional reaction to the reality situation of the barren state In the evaluation of individual cases it is difficult for the gynecologist to separate cause from effect In dealing with any deep seated emotional problems associated with in fertility the role of the psychiatrist is of evident importance Researchwise the psychosomatic factors in infertility are worthy of increased attention from both gynecological physiologist and psychiatrist alike

The purpose of an infertility investigation is two fold Primarily the physician is concerned with help ing the couple in reaching their ultimate goal that of a successfully terminated pregnancy Equally as important is his ability to intelligently offer them a prognosis so that they might plan their lives and perhaps consider adoption at an earlier stage in their marriage Because of the inadequacies of present methods of diagnosis it is difficult to predict the chances for success save in those few cases where sterility can be adjudged absolute The inadequacies of therapy for infertility are evident Some progress though still unsatisfactory by any standards has been achieved *in the treatment of disorders of ovulation* and anatomical tubal pathology Treatment of male infertility remains discouragingly unrewarding The problem has been aptly characterized by C Lee Buxton who has stated[19] Certainly the field of human reproduction presents a vast unexplored area awaiting the assault of the inquisitive and imagina tive mind It would appear that research in this field has now reached the point where the clinician must

largely give way to the physiologist the chemist the endocrinologist the immunologist the geneticist *for the sake of obtaining further vital information*

BIBLIOGRAPHY

1 Abarbanel A R 1948 Artificial reproduction of the cyclic changes in cervical mucus in human castrates West J Surg 56 26 34

2 Abu Hayder W Laidlaw J C Nusimovich B and Sturgis S 1951 Hyperadrenalism and the Stein Leventhal syndrome J Clin Endocrinol & Metab 14 766

3 Asherman J G 1952 X ray therapy in sterility Gynecologia 133 65 73

4 Bailey K U 1937 The operation of extroversion of the ovaries for functional amenorrhea especially of the secondary type J Obst & Gynaec Brit Emp 44 637-49

5 Balboni G 1954 Richerche istochimiche sull epitelio tubarica della donna Riv Obstet e Gin 9 164 94

6 ——— 1954 Lo stroma tubarico in rapporto al ciclo mestruale ed al momento istofunzionale delle cellule dell epitelio Riv di Biologia 47 153 68

7 Bang J 1950 Complications of hysterosalpingography Acta Obst et Gynec Scandinav 29 383 99

8 Bayle H 1950 Masculine sterility latero lateral epididymodeferens anastomosis in azospermia by obliteration statistics in ninety five surgically explored cases Urol and Cutan Rev 54 129 35

9 Bendick A J 1954 A simplified inexpensive technique in hysterosalpingography Surg Gynec & *Obst 99 642 6*

10 Benson R C and Daily M E 1955 The menstrual pattern in hyperthyroidism and subsequent post therapy hypothyroidism Surg Gynec & Obst 100 19 26

11 Bergman P 1950 Sexual cycle time of ovulation and of optimal fertility in women Studies on basal body temperature endometrium and cervical mucus Acta Obst et Gynec Scandinav 29 1 139

12 Bloom E 1950 A one minute live dead sperm stain by means of eosin nigrosin Fertil & Steril 1 176-7

13 Bloomfield A 1946 Six cases of venous intravasation following intrauterine lipiodal injection J Obst & Gynaec Brit Emp 53 345-6

14 Bromberg Y M and Bercovici B 1956 Occult intermenstrual bleeding about the time of ovulation Fertil & Steril 7 71 9

15 Brown W E and Bradbury J T 1950 Gonadotropins in gynecology Progress in Gynecology Vol II edited by Meigs J V and Sturgis S H Grune and Stratton New York pp 261 9

16 Brown W E Jennings A F and Bradbury J T 1949 The absorption of radiopaque substances used in hysterosalpingography *Am J Obst & Gynec 58 1041 53*

17 Buxton C L Personal communication

18 ——— 1950 The atypical secretory phase Menstruation and Its Disorders edited by Engle E T Charles C Thomas Springfield Ill pp 270 88

19 ——— 1956 Human infertility Am J M Sc 231 694 700

20 Buxton C L and Engle E 1950 Time of ovulation correlation *between basal temperature* appearance of the endometrium and appearance of the ovary Am J Obst & Gynec 60 539 51

21 Buxton C L and Herrmann W L 1954 Effect of thyroid therapy on menstrual disorders and sterility *J A M A 155 1035-9*

22 Buxton C L and Southam A 1955 A critical survey of present methods of diagnosis and therapy in human infertility Am J Obst & Gynec 70 741 9

23 ——— 1956 Ovarian dysfunction as an infertility problem Obst & Gynec 8 135 9

24 Buxton C L Southam A Herrmann W Girvin C and Nadel H 1951 Bacteriology of the cervix in human sterility Fertil & Steril 5 493 514

25 Buxton C L and VandeWiele R 1954 Wedge resection for polycystic ovaries New Eng J Med 251 293 7

26 Buxton C L and Wong A S H 1952 Spermicidal *bacteria in the cervix as a cause of sterility* Am J Obst & Gynec 64 628 33

27 Charny C W *1956* Treatment of male infertility with large doses of testosterone J A M A 160 98 101

28 Clift A and Hart J 1954 Post-coital tests on a statistical basis Fertil & Steril 5 544 50

29 Cohen M R and Stein I F 1951 Sperm survival at estimated ovulation time 1951 Fertil & Steril 2 20 27

30 Cohen M R and Stein I F Sr and Kaye B M 1953 Spinnbarkeit a characteristic of cervical mucus Fertil *& Steril 3 201 8*

31 Collins C J 1950 Low dosage pituitary ovarian irradiation in secondary amenorrhea *and associated* sterility Southern Med J 43 527 31

32 Comminos A C 1955 Thyroid and reproductive ability Obst & Gynec 7 260 66

33 *Corner G W Sr Farris E J* and Corner G W Jr 1950 The dating of ovulation and other ovarian crises by histological examination in comparison with the Farris test Am J Obst & Gynec 59 514 28

34 Da Paz A C 1952 Partial tubal obstruction Rev brasil cir 24 407 34

35 Davis M E 1946 Clinical use of oral basal temperatures *J A M A 130 9-9 32*

36 Decker A 1952 Culdoscopy—A New Technique in *Gynecologic and Obstetric Diagnosis* W B Saunders and Co Philadelphia

37 Decker A and Cherry T 1944 Culdoscopy—a new method in the diagnosis of pelvic disease—preliminary report Am J Surg *64 40 44*

38 Decker A and Decker W H 1954 A tubal function test Obst & Gynec 4 35-8

39 Escamilla R F and Lisser H 1942 Simmonds disease J Clin Endocrinol 2 65 96

40 Farris E J 1946 A test for determining the time of ovulation and conception in women Am J Obst & Gynec 52 19 27

41 Finegold W J 1956 Cortisone in sterility Fertil & Steril 7 28 30

42 *Fischer R H 1954 Progesterone* metabolism III Basal body temperature as an index of progesterone production and its relationship to urinary pregnandiol Obst & Gynec 3 615 26

43 Getzoff P L 1955 Clinical evaluation of testicular biopsy and the rebound phenomenon Fertil & Steril 6 465 74

44 Gillam J S 1955 Study of the inadequate secretion phase endometrium Fertil *& Steril 6 18 36*

45 Goldhar A Grody M H and Masters W H 1952 The vaginal smear as an ovulatory index Fertil & Steril 3 376 92

46 Goldsmith R E Sturgis S H Lerman J and Standbury J B 1952 Menstrual pattern in thyroid disease J Clin Endocrinol 12 846-55

47 Greenblatt R B Barfield W E Lampros C P 1956 Cortisone treatment in infertility Fertil & Steril 7 203 12

48 Greenhill J P 1937 Evaluation of salpingostomy and tubal implantation for the treatment of sterility Am J Obst & Gynec 33 39 51

49 —— 1953-54 Yearbook of Obstetrics and Gynecology The Yearbook Publishing Co Chicago pp 308 9

50 Guillemin R and Rosenberg B 1955 Humoral hypothalamic control of anterior pituitary A study with combined tissue cultures Endocrinology 57 599 607

51 Holmstrom E G 1954 Progesterone treatment of anovulatory bleeding Am J Obst & Gynec 68 1321 9

52 Horne H W and Abu Hayder W 1956 Adrenal hyperactivity and infertility Proc Second World Congress on Fertility and Sterility In press

53 Hotchkiss R S 1944 Fertility in Men J P Lippincott Co New York pp 117-30

54 Hotchkiss R S Asdrubal B P and Kleegman S 1955 Artificial insemination with semen recovered from the bladder Fertil & Steril 6 37-42

55 Ingersoll F M and McDermott W V Jr 1950 Bilateral polycystic ovaries Stein Leventhal syndrome Am J Obst & Gynec 60 117 25

56 Israel S L 1952 The empiric usage of low dosage irradiation in amenorrhea Am J Obst & Gynec 64 971-87

57 Jones G E S Howard J E and Langford H 1953 The use of cortisone in follicular phase disturbances Fertil & Steril 4 49 59

58 Kalant N Pattee C J Simpson G W and Hendelman M 1956 Timing of ovulation Fertil & Steril 7 57 65

59 Kaplan I I 1950 The question of genetic injury following x ray irradiation of the ovaries in the treatment of sterility J Ob t & Gynaec Brit Emp 57 767 79

60 —— 1956 An analysis of 530 pregnancies following x ray therapy of infertile married women and a follow up report on children born to them Proc Second World Congress on Fertility and Sterility In press

61 Kelly J V and Rock J 1956 Culdoscopy for diagnosis in infertility Am J Obst & Gynec 72 523 7

62 Kleinfelter H F Albright F and Griswold G C 1943 Experience with a quantitative test for normal or decreased amounts of follicl stimulating hormone in the urine in endocrinological diagnosis J Clin Endocrinol 3 529-44

63 Kneer M Burger H and Simmer H 1952 Über die Atmung der Schleimhaut menschlicher Eileiter Arch Gynak 181 561 74

64 Kneer M and Cless H 1951 Flimmerung und Stromung im menschlichen Eileiter Geburtsh u Frauenh 11 233-9

65 Kroger W S and Freed S C 1950 Psychosomatic aspects of sterility Am J Obst & Gynec 59 867 74

66 —— 1951 Psychosomatic Gynecology Saunders Philadelphia pp 28_ 94

67 Lampe E H and Masters W H 1956 Problems of male fertility II Effect of frequent ejaculation Fertil & Steril 7 123-32

68 Lerman J 1938 The thyroid in gynecology Progress in Gynecology vol II edited by Meigs J V and Sturgis S H Grune and Stratton New York pp 253-6

69 Leventhal M L and Cohen M L 1951 Bilateral polycystic ovaries the Stein syndrome Am J Obst & Gynec 61 1034-44

70 Levin L Buxton C L and Engle E T 1949 On the validity of the hyperemia method for determining ovulation time in women Am J Obst & Gynec 58 795-8

71 MacLeod J 1956 Human semen Fertil & Steril 7 368-86

72 MacLeod J and Gold R Z 1951 The male factor in fertility and infertility II Spermatozoan counts in 1000 men of known fertility and in 1000 cases of infertile marriage J Urol 66 436-49

73 —— 1951 The male factor in fertility and infertility IV Sperm morphology in fertile and infertile marriage Fertil & Steril 2 394-414

74 —— 1953 The male factor in fertility and infertility VI Semen quality and certain other factors in relation to ease of conception Fertil & Steril 4 10-33

75 MacLeod J Gold R Z and McLane C M 1955 Correlation of the male and female factors in human infertility Fertil & Steril 6 112-41

76 MacLeod J and Hotchkiss R S 1942 The distribution of spermatozoa and of certain chemical constituents in the human ejaculate J Urol 48 225 9

77 Manzi L 1947 Cited by Rubin I C in Uterotubal Insufflation C V Mosby Co St Louis p 126

78 Mastroianni L Laberge J L and Rock J 1957 Appraisal of the efficacy of artificial insemination using husband s sperm and e aluation of insemination techniques Fertil & Steril 8 260 66

79 Mastroianni L and Rock J 1957 Introital obstruction to intromission treatment and results Obst & Gynec 9 55-7

80 McGavack T H 1951 The Thyroid C V Mosby Co St Louis pp 399-401

81 Michelson L 1946 Vasoepididymal anastomosis by production of permanent fistula with use of stainless steel wire Surg Gynec & Obst 82 327-31

82 Muller H J 1952 Damage to potency caused by irradiation of the gonads Am J Obst & Gynec 67 467 79

83 Mulligan W J Rock, J and Easterday C L 1953 Use of polyethylene in tuboplasty Fertil & Steril 4 428-33

84 Murphy D P and Farris E J 1947 Conception following the prediction of the day of ovulation with the rat test Am J Obst & Gynec 54 467 74

85 Murray E 1953 The peritoneal factor in female sterility Fertil & Steril 4 371-83

86 Novak E 1952 Gynecologic and Obstetric Pathology 3rd ed Saunders Philadelphia p 279

87 Novak E and Everett H S 1928 Cyclical and other variations in tubal epithelium Am J Obst & Gynec 16 499 530

88 Noyes R W and Haman J O 1953 Accuracy of endometrial dating Fertil & Steril 4 504 17

89 Noyes R W Hertig A T and Rock J 1950 Dating the endometrial biopsy Fertil & Steril 1 3-25

90 Page E W 1952 Discussion of paper by Israel S L Am J Obst & Gynec 64 983

91 Papanicolaou G N 1933 The sexual cycle in the

human female as revealed by vaginal smears Am J Anat 52 519 616
92 Papanicolaou G Traut H F and Marchetti A 1948 The epithelia of woman s reproductive or gans a correlative study of cyclic changes Com monwealth Fund New York
93 Patten B M 1946 Human Embryology The Blak iston Co Philadelphia p 84
94 Payne S 1952 Use of low dosage irradiation in the treatment of infertile women with ovarian dys function Fertil & Steril 3 500 526
95 Payne S and Skeels R F 1954 Fertility as eval uated by artificial insemination Fertil & Steril 5 32 9
96 Reifenstein E C 1946 Psychogenic or hypothala mic amenorrhea M Clin North America 30 1103 14
97 Research Correlating Committee of the A S S S 1951 Evaluation of the barren marriage Fertil & Steril 2 1 14
98 Reycraft J L 1949 Operative procedures for the treatment of sterility and ovarian dysfunctions Am J Obst & Gynec 57 1069 74
99 Rock J Personal communication
100 ——— 1948 Discussion of paper by TeLind R W and Ruthdge F Am J Obst & Gynec 55 115
101 ——— 1948 Investigation and treatment of infertil ity M Clin North America 32 1171 93
102 ——— 1950 Causes and relief of infertility Progress in Gynecology vol II edited by Meigs J V and Sturgis S H Grune and Stratton New York p 320
103 ——— 1950 Progress in Gynecology vol II edited by Meigs J V and Sturgis S H Grune and Strat ton New York p 321
104 ——— 1955 Discussion of paper by Buxton C L and Southam A Am J Obst & Gynec 70 752
105 Rock J Bartlett M K Gould A G and Ruther ford R W 1940 The effect of subcastrative roent gen therapy on ovarian physiology Surg Gynec & Obst 70 903 13
106 Rock J and Hertig A T 1942 Some aspects of early human development Am J Obst & Gynec 44 973 83
107 ——— 1944 Information regarding the time of hu man ovulation derived from study of three unfer tilized and eleven fertilized ova Am J Obst & Gynec 47 343 56
108 Rock J and Mulligan W J 1956 Data presented at the annual meeting of the Conn Society of Amer ican Board of Obstetricians and Gynecologists Inc
109 Rock J Mulligan W J and Easterday C L 1954 Polyethylene in tuboplasty Obst & Gynec 3 21 9
110 Roland M 1952 A simple test for the determina tion of ovulation estrogen activity and early preg nancy using the cervical mucus secretion Am J Obst & Gynec 63 81 9
111 Roland M Carpenter F Rich J 1953 A new water soluble medium for the study of hysterograms and hysterosalpingograms Am J Obst & Gynec 65 81 7
112 Rubin I C 1920 The non operative demonstration of patency of the Fallopian tubes J A M A 75 661 6
113 ——— 1952 Comparison of carbon dioxide and opaque media in the diagnosis of tubal pathology Fertil & Steril 3 179 87
114 ——— 1952 Third generation follow up in women receiving pelvic irradiation J A M A 150 207 9
115 Rubin I C 1952 The non-operative determination of patency of Fallopian tubes Am J Med 13 562 9
116 ——— 1954 Differentiation between uterus and tubes as cause of oscillations recorded during uter otubal insufflation Fertil & Steril 5 147 67
117 ——— 1954 Uterotubal insufflation Value in the treatment of tubal obstruction to ovular migration Fertil & Steril 5 311 23
118 Rubin I C Myller E Hartman C G 1953 Sal pinx a new approach to the ideal radiopaque me dium for hysterosalpingography Fertil & Steril 4 357 70
119 Rugh R 1952 Cited by Buxton C L in discus sion of paper by Payne S Fertil & Steril 3 525
120 Russel K P 1954 The current status of the pro tein bound iodine in obstetrics and gynecology Obst & Gynec Surv 9 157 74
121 Russell W L 1952 Letter to the editor J A M A 150 1619 20
122 Sechinger D L and Snyder F F 1926 Cyclic changes in the spontaneous contractions of the hu man Fallopian tubes Bull Johns Hopkins Hosp 39 371 8
123 Rutherford R N 1944 Physiologic intermenstrual bleeding—gross or microscopic—as a possible diag nostic aid in abdominal pain studies West J Surg 52 62 8
124 Schaffer S 1953 Female genital tuberculosis Obst & Gynec Surv 8 461 501
125 Sharman A 1952 Genital tuberculosis in the fe male J Obst & Gynaec Brit Emp 59 740 42
126 ——— 1954 Some lessons from 4000 uterotubal in sufflations Brit M J 1 239 42
127 Sheehan A L 1954 The incidence of post partum hypopituitarism Am J Obst & Gynec 68 202 21
128 Sheffery J B 1954 A new method of determining tubal patency and tubal ciliary activity South M J 47 221 4
129 Shettles L B 1953 Observations on human fol licular fluid and tubal ova Am J Obst & Gynec 66 235 53
130 ——— 1955 A morula stage of human ovum devel oped in vitro Fertil & Steril 6 287 9
131 Shorr E 1940 A new technique for staining vag inal smears Science 91 321 2
132 ——— 1940 A new technique for staining vaginal smears Science 91 579 80
133 ——— 1941 A new technique for staining vaginal smears III A single differential stain Science 94 545 6
134 Siegler S L 1944 Fertility in Women J P Lip pincott Co Philadelphia p 103
135 Simmons F 1950 Progress in Gynecology vol II edited by Meigs J V and Sturgis S H Grune and Stratton New York pp 335 6
136 Sims J M 1866 Clinical Notes on Uterine Sur gery Robert Hardwicke London pp 361 2
137 ——— 1868 Illustrations of the value of the micro scope in the treatment of the sterile condition Vol 2 465 6 492 4
138 Sjovall A 1938 Untersuchungen uber die Bedeu tung der Ovarialhormon fur den Cyklus der Cer vixschleimhaut des Meerschweinches Acta Obstet et Gynec Scandinav 18 (supp 4) 105 52
139 Southam A L and Buxton L 1956 Seventy post coital tests made during the conception cycle Fer til & Steril 7 133 9
140 Stabile A 1954 Interpretation of manometric os cillations observed during uterotubal insufflation Fertil & Steril 5 138 46

141 Stein I F Sr 1955 The management of bilateral polycystic ovaries Fertil & Steril 6 190 202
142 Stein I F and Leventhal M L 1935 Amenorrhea associated with bilateral polycystic ovaries Am J Obst & Gynec 29 181 91
143 Swartz D P and Jones G S 1956 Clinical observations on the role of progesterone in anovulatory uterine bleeding Presented at the Twelfth Annual Conference of the A S S S Chicago
144 Swyer G I M 1953 Section of urology Discussion on male infertility Proc Roy Soc Med 46 835-8
145 Tietze C 1956 Statistical contributions to the study of human fertility Fertil & Steril 7 88 95
146 Tompkins P 1944 Use of basal temperature graphs in determining date of ovulation J A M A 124 698 700
147 —— 1953 Endometrial biopsy determination of incidence of ovulation in 402 regularly menstruating women Fertil & Steril 4 76 9
148 Turner H H 1938 A syndrome of infantilism congenital webbed neck and cubitus valgus Endocrinology 23 566 74
149 Tyler E T 1953 The thyroid myth in infertility Fertil & Steril 4 218 32
150 —— 1953 Physiological and clinical aspects of conception J A M A 153 1351 6
151 Tyler E T and Singher H O 1956 Male infertility—status of treatment prevention and current research J A M A 169 91 7
152 Vessel M 1954 Multiple uterotubal insufflations in cases of sterility due to tubal occlusion Am J Obst & Gynec 68 810 14
153 Vogt C J 1956 Hystero alpingography—a safe diagnostic and therapeutic gynecological procedure Am J Obst & Gynec 67 1298 1305
154 Weir W C Jefferies W McK Kent G T and Wier D R 1954 Booster estrogen therapy in infertility Am J Obst & Gynec 67 585 95
155 Weisman A I 1951 The volumetric capacity of the human nulliparous uterus Am J Obst & Gynec 61 202 4
156 —— 1952 Incidence of residual intraperitoneal iodochloral after hysterosalpingography Fertil & Steril 3 290 96
157 Westman A 1937 Investigations into transit of ova in man J Obst & Gynaec Brit Emp 44 821 38
158 —— 1942 Investigations into the transport of the ovum Studies on Testes and Ovary Eggs and Sperm edited by Engle E T Thomas Springfield Ill pp 163 8
159 Whitelaw M J 1950 Use of the cervical cap to increase fertility in cases of oligospermia Fertil & Steril 1 33 9
160 —— 1955 What is normal female fertility? Fertil & Steril 6 103 7
161 Wilhelm S F and Marks G 1955 Cortisone and adrenalectomy in treatment of sterility in women with adrenogenital syndrome J Urol 73 17 24
162 Wilkins L Crigler J F Silverman S H Gardner L I Migeon C J 1952 Further studies on the treatment of congenital adrenal hyperplasia with cortisone J Clin Endocrinol 12 277 95
163 Wilkins L Grumback M M and VanWyck J S 1954 Chromosomal sex in ovarian agenesis J Clin Endocrinol 14 1270 71
164 Wilkins L Lewis R A Kleon R Gardner L I Crigler J F Jr Rosenberg E and Migeon C L 1951 Treatment of congenital adrenal hyperplasia with cortisone J Clin Endocrinol 11 1 25
165 Williams W W and Pollak O J 1950 Study of sperm vitality with the aid of eosin nigrosin stain Fertil & Steril 1 178 81
166 Wilson L 1954 Ovarian dysfunction Obst & Gynec 4 97 104
167 Wittkower E and Wilson A T M 1940 Dysmenorrhea and sterility personality studies Brit M J 2 586-90
168 Woltz J H E Bradford W Z Bradford W B and Brown C W 1953 The treatment of infertility in private practice with emphasis on hysterosalpingography Am J Obst & Gynec 66 801 7
169 Zondek B Bromberg Y M and Polishuk Z 1948 Constant oligospermia and periodic oligospermia Am J Clin Path 18 874 8

IX

The Endocrine Pathology of the Female Reproductive System

WILLIAM B OBER M D

This is the warehouse of the world's chief trade
On this soft anvil all mankind was made
JOHN WILMOT EARL OF ROCHESTER

IT IS NOT possible in a single chapter to deal exhaustively with the entire morbid anatomy of the female reproductive organs Adequate presentations of this subject are available in standard texts notably those by Novak and Herbut It will be assumed in this chapter that the reader is familiar with the general pathology of the female genitalia and the exposition will be limited to those topics in which endocrine phenomena play a dominant role and those which the impact of hormones upon end organs is significant Documentation will be limited to pertinent references and illustrations will be utilized only when such illustrations are not readily available in general texts

The principal limiting factor in our understanding of hormones in relation to the tissues of the female genitalia is that we cannot assign with certainty the presence of a specific hormone in a specific cell *at a specific time in a specific quantity* With the exception of pituitary and chorionic gonadotropins the hormones involved are all steroids The differences in tissue responses depend upon various atoms tacked onto the familiar steroid ring at different positions Our present day information about how different steroid molecules are synthesized how one hormone may be converted into another both in terms of the biochemical process and the cell in which the reaction occurs is meager Until such information becomes available it is difficult to relate a specific hormone to a specific response in the cells of an end organ End organs can react in a limited number of ways and the number of stimulating substances is larger than the number of responses It is quite understandable that a comparable response of tissue can be obtained through a variety of pathways

The principal changes in end organ tissues are atrophy hypoplasia hyperplasia or hypertrophy metaplasia and neoplasia While in experimental situations it is possible to produce a specific reaction classifiable as one of these changes by the administration or abolition of one hormone this is rarely the case in clinical medicine In the usual human patient one perforce deals with more complex situations in which more than one hormone is involved Not only is the quantity of the hormones significant but their relationship to other hormones determines the clinical picture and the anatomic changes Nor can we assess with accuracy in most cases the role of associated nonendocrine factors in the production of lesions in the human female

I VULVA AND VAGINA

The squamous epithelium which lines the mucous membranes of the vulva and vagina is an end organ for estrogenic substances The embryologic consideration that the lower two thirds of the vagina are derived from the ectoderm of the urogenital sinus and the upper third is derived from the Mullerian apparatus does not appear to influence the nature of the response At birth the stratified squamous epithelium is from six to ten cells thick During the week after birth it becomes thinner by half as the effect of maternal or placental estrogen upon the tissues of the infant is abolished It remains in this thinned state during childhood becomes thicker at puberty varies somewhat with the menstrual cycle and with pregnancy and atrophies after the menopause to its previous thinned state During active sexual life i e under normal estrogen stimulation it is cornified but not keratinized During states of hyperestrogenism increased cornification can be demonstrated by exfoliative cytology and the converse obtains for hypoestrogenism During the proliferative (follicular) phase of the menstrual cycle the superficial cells become larger and more flattened the cytoplasm showing increasing acidophilia the nuclei becoming pyknotic During the secretory (luteal) phase the superficial cells become irregular often elongated with a tendency for folding of the edges and curling Cytoplasmic basophilia develops progressively but nuclear pyknosis is constant The presence of cytoplasmic granules is no-

ticeable these are usually acidophilic and refractile As the expected time for menstruation approaches cytoplasmic basophilia becomes predominant and nuclear enlargement develops While these minute changes are relatively constant and easily reproducible in smears studied from a large number of women they are of limited diagnostic value they merely corroborate readily ascertainable clinical data

The only two clinical situations in which the relationship of estrogens to the vulvovaginal mucosa is significant is in vulvovaginitis puellarum and in senile vaginitis In both conditions the common denominator is the thinning of the squamous epithelium in association with low estrogen production It is probable that a thin mucosa is more susceptible to infection than a thick one However the offending organisms are usually of low virulence even saprophytic and such factors as symbiotic organisms changes in pH of vaginal secretions etc must be considered in evaluating these infections Before the era of antibiotics it was customary to treat vulvovaginitis puellarum with estrogen preparations in an effort to thicken the squamous epithelium this was moderately successful Estrogen therapy is still used in stubborn cases In contrast senile vaginitis usually responds poorly to antibiotics either systemic or local and estrogen therapy is universally recommended

It is of more than passing interest to observe that vulvovaginitis puellarum is often caused by a gram negative intracellular diplococcus Formerly it was generally thought that such infections were gonorrheal and strenuous efforts were made to ascertain possible venereal exposure in little girls of four five or six years of age Weaver[65] has shown by careful bacteriologic study that the chief offending organism was *Neisseria sicca* a normal inhabitant of the oropharynx Presumably such childhood infections are incurred by digital contamination from the oral and nasal passages

Recently estrogenic creams have been used successfully to treat labial adhesions in infants This effect also depends on increasing the thickness of the stratified squamous epithelium

An infrequent anomaly is the occurrence of ectopic mammary tissue in the labium majus in the caudad extension of the ventral milk line On occasion such tissue responds to the menstrual cycle growing turgid and tender as menstruation approaches On rare occasions such tissue will lactate after delivery

II CERVIX UTERI

Much like the squamous epithelium of the vagina that of the cervix can respond in a limited way to estrogenic stimulation However the anatomic observations involve a greater degree of complexity for instead of there being only one cell type as in the vaginal epithelium the cervix contains three cell types the squamous cell the reserve cell and the columnar cell Proliferation of all of these cell forms can be induced by estrogens and in certain situations the morphologic demonstration can be striking Chronologically the congenital erosion seen at the cervical os of newborn infants is the earliest example This innocuous lesion represents merely a transitional zone from the orderly stratified squamous epithelium of the exocervix to the orderly branched tubular glands of the endocervix In the zone of transition there is metaplastic squamous epithelium and below the squamous cells a layer of proliferated reserve cells can often be distinguished

Morphologically indistinguishable phenomena can be seen as reparative processes in inflammatory lesions of the cervix during adult life Carmichael and Jeafferson were among the first to emphasize the prominence of reserve cells in squamous metaplasia of the cervix Hellman *et al*[17] have summarized the evidence for the importance of estrogens in this type of metaplastic response pointing out in addition that similar changes in the cervix have been seen in association with granulosa cell tumors of the ovary It seems probable that estrogenic substances can be under special condition a determining factor in the maturation of reserve cells into squamous cells albeit such estrogen stimuli need not initiate the response

Bridging the gap between metaplasia and neoplasia are the lesions formerly referred to as condyloma of the cervix and more recently squamous papilloma Morphologically they range from hypertrophic epithelium resting on a polypoid fibrous base characterized by thickening of the entire epithelium prolongation and widening of the rete pegs and increased mitotic activity to truly papilliferous lesions in which hyperplastic epithelium is supported by branching cores of fibrovascular stroma The commonest occasion for the development of these growths is pregnancy they arise toward the end of the first trimester increase moderately in size until term regressing after delivery As is evident from their gross appearance they are friable and may occasion vaginal spotting Edmondson *et al*[9] have described in some detail the typical squamous papillomas of pregnancy Greene and Peckham[13] have described examples both during pregnancy and in the absence of pregnancy They point out that the covering epithelium may exhibit a wide range of deviations from the norm Particularly in pregnancy cellular atypism can be a prominent feature and mistaken diagnoses of carcinoma *in situ* made

The effects of the endocrine stimuli of pregnancy upon the morphology of the squamous epithelium

Biopsies taken after pregnancy in all these cases (Figs 1 2 3) showed reversal of the cellular changes



Fig 1 Cervix in pregnancy The presence of bizarre large cells some with hyperchromatic nuclei suggests malignancy × 100



Fig 2 Cervix in pregnancy The ballooned cells with cytoplasmic hydrops and nuclear pyknosis are atypical × 165

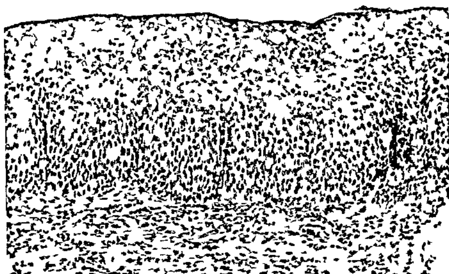


FIG 3 CERVIX IN PREGNANCY The surface epithelium is thickened and there is hydrops of the superficial layers There is hyperplasia of the basal layers and the long axis of many cells is perpendicular to the basement membrane However a degree of maturation is maintained Mitotic figures are numerous × 125

of the cervix have received considerable attention in recent years particularly since the recent impetus given to the early detection of carcinoma in the pre invasive stage (Figs 1 2 3) It is well within the experience of every pathologist to receive biopsy material from the cervix in a pregnant woman which is both confusing and disturbing Loss of cellular polarity the presence of cells with large hyper chromatic nuclei and increased mitotic activity are common features Experience with such lesions whether associated with truly papillomatous growths or merely on a flat surface has shown that for the most part such lesions are innocuous will regress after pregnancy and do not portend the development of a carcinoma invasive or in situ Conservatism both in diagnosis and in management cannot be urged too strongly pending more specific information regarding the mechanism by which such changes occur It is safe to implicate estrogenic stimulation as being involved to some degree but the role of other hormones is as yet undetermined It must be recognized that such changes occur in a very small minority of women and need not recur in subsequent pregnancies in the same woman Although the significance of this fact is not clear it must be apparent that some other stimulus for the unusual changes in the appearance of the epithelial cells must be present (Fig 4)

To a lesser degree the columnar cells of the cervix respond to endocrine stimuli The increased secretion of endocervical mucus during pregnancy and other hyperestrogenic states is well known Anatomically there is a true hyperplasia of the glandular epithelium but it is of no great degree Likewise the endocervical stroma respond to hormones even as the endometrial stroma albeit to a lesser degree Decidual reactions in pregnancy can be detected in the majority of cases if sufficient tissue is obtained for microscopic examination On occasion the decidual transformation may be pronounced associated with a cervical erosion and produce vaginal spotting Like the decidual reaction elsewhere it regresses when the stimulus is withdrawn

The relationship of hormones to cervical cancer in the human female is obscure Certainly there is no body of evidence to implicate estrogens to the same extent in pathogenesis as in endometrial cancer It is tempting to generalize that the development of squamous carcinomas is more likely to be associated with exogenous or environmental factors and the development of glandular carcinomas more likely to be associated with endogenous occasionally endocrine factors Like most generalizations in oncology the exceptions are so numerous and the applications so few that they lose force However Hertz *et al*[25] have utilized progesterone as an adjuvant to the management of squamous carcinoma of the cervix proceeding on the observation that progesterone can reverse many of the estrogen induced atypicalities of nonneoplastic cervical squamous epithelium They noted a moderate degree of regression in about 60 per cent of the cases but examination of sub-

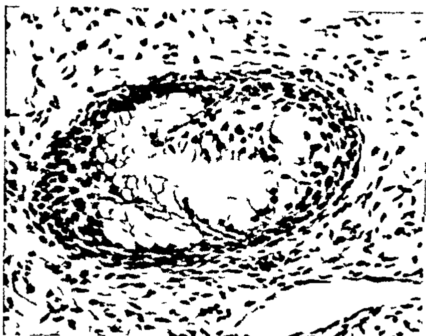


FIG 4 ENDOCERVICAL GLAND SHOWING SQUAMOUS METAPLASIA OF RESERVE CELL TYPE Estrogens promote and enhance this phenomenon ×200

sequently excised tissues showed no specific change in the tumor cells

In only one respect does the absence of hormonal stimuli affect cervical neoplasia Study of cervical biopsies shows that in postmenopausal women a diagnosis of carcinoma *in situ* is never difficult or borderline granted adequate tissue for examination The facility of diagnosis and the unequivocal nature of the morphologic changes is in striking contrast to the rather frequent instances of doubtful lesions encountered in routine pathologic practice in premenopausal women I can offer no explanation why this should be so

III CORPUS UTERI

A Endometrium

The endometrium is the most important end organ in the female genital tract It is composed of simple tubular glands lined by a columnar epithelium supported by a highly specialized stroma These structures are all derived from the Mullerian apparatus and represent a principal contribution of the mesenchyme of the urogenital ridge to the reproductive tract Both glands and stroma are controlled by the cyclic elaboration of estrogenic substances and progestational substances The ability to respond is not confined to the period of active sexual life Secretory vacuoles and decidual transformation have been observed in the endometria of the newborn a response to stimulation from maternal hormones which traverse the placental membrane Menstruatio praecox occurs in these infants within the first few days of life as the maternal hormones are metabolized thereby producing a withdrawal effect During childhood the endometrium is inactive only to become responsive at menarche as the ovarian cycle commences (Figs 5 6)

The specific effects of estrogenic stimulation are proliferative i e the repair of the endometrium after menstruation and the continued growth of glands and stroma are promoted entirely by estrogens Only after ovulation are the effects of progesterone observed these include glandular secretion and decidual transformation of the stroma

Recently McKay Hertig Bardawil and Velardo[39] reported that alkaline phosphatase is maximally present during the proliferative (estrogenic) phase and acid phosphatase is present maximally during the progravid (luteal) phase inferring that the enzyme systems which control carbohydrate metabolism by endometrial glandular epithelium are controlled cyclically by the steroid hormones

The specific histologic details are clearly set forth by Hertig and his associates Basing their timetable on the hypothetical 28-day cycle they point out that such proliferative phenomena of estrogen stimulation as mitotic activity in both glandular epithelium and stromal cells and pseudostratification of glandular epithelium are maximal at mid cycle (the mean time for ovulation) The phenomena of progesterone stimulation occur in the latter half of the cycle secretory vacuolation at the base of glandular cells first appears about 48 hours after ovulation and over the next few days the products of secretion

migrate toward the glandular lumen and are discharged into it regression of secretory activity being perceptible by the 23d day of the cycle At this time stromal edema reaches its maximum and the predecidual transformation of the endometrial stroma is first visible about the spiral arterioles at this time Predecidual transformation increases from the 23d to the 28th day as the corpus luteum discharges its most active constituents This elaborate and complex sequence of histologic events is adaptive it is designed to receive a fertilized ovum if none is received there is no additional stimulus from the primitive trophoblast and menstruation occurs If the sin of teleology is venial menstruation can be called the tears of the frustrated endometrium The important dates in the hypothetical 28 day cycle can be enumerated Day 1 onset of menstruation Day 14 ovulation corpus luteum begins to form Day 16



Fig 5 Endometrium of newborn showing secretory activity × 115

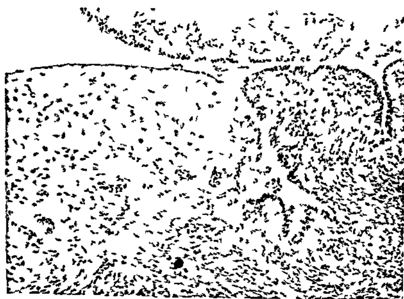


Fig 6 Endometrium of newborn showing decidual transformation of stroma × 100

secretory vacuoles in glands Day 23 beginning of predecidual response beginning of regression of corpus luteum

In practice the application of such a schedule must be tempered by the recognition that the range of variability even in normal women can be considerable and in women with menstrual disorders or problems of infertility even greater Bartelmez[2] has *emphasized this point* amplifying it with accurate statistical analysis He states that the morphologic changes in the ovaries provide the only satisfactory criteria for determining the sequence of *events during the cycle This is especially true of* the meteoric career of the corpus luteum The target organs are less satisfactory for they are subject to other factors than hormonal tides It is unfortunate that there *is no convenient technique for direct* evaluation of the corpus luteum which can be applied to problems in infertility Until such methods are developed the endometrial biopsy will remain the principal method for evaluating the morphologic status of reproductive capacity and it is important to recognize its limitations

In general microscopic examination of an endometrial biopsy can furnish satisfactory evidence that ovulation has occurred only rarely is the presence of secretory vacuolation misleading Biopsies taken at or just before the expected time of flow are useful in evaluating the quantity of the predecidual response However caution in interpretation must be urged for even in one cycle there may be significant morphologic variation from one part of the endometrium to another Any pathologist who has taken multiple sections from separated areas of uterine mucosa in hysterectomy specimens will appreciate this point Consequently it is unwise to date a small biopsy too rigidly Optimal results in fertility *studies depend upon frequent consultation between* the pathologist and the clinician both in respect to establishing the patient s normal pattern of response and in evaluating the results of therapy By the same token it is incumbent upon the clinician to inform the pathologist of such important data as the date of the last menstrual period the changes in the temperature the specific hormones used in treatment their dosages and the time during the cycle they were administered It need scarcely be mentioned that the endometrial biopsy is useful in ruling out intrinsic causes for infertility such as tuberculous endometritis hyperplasia neoplasia etc

It is evident that a biologic system which depends upon the sequential elaboration of two hormones which have reciprocal action upon a common end organ is a delicately balanced phenomenon depending upon *appropriate* elaboration of each substance in respect to time and to quantity as well as upon the receptivity of the end organ If we assume that there are 13 menstrual periods a year and that an average woman can have periods from the age of 12 to the age of 48 the endometrial cycle is repeated 468 times not allowing for periods missed because of pregnancy Even under perfectly normal conditions the probability of biologic variation is such that almost every woman will experience at some time a disturbance of cycle which may necessitate medical attention One of the most convenient fictions in contemporary gynecology is the 28 day cycle Every obstetrician and gynecologist is familiar with the voluntary statement My periods were always regular like clockwork every 28 days until There is little accuracy of mensuration in respect to menstruation It must be observed however that were a patient to tell her physician that *her last 10 periods had occurred at a mean interval* of 26 4 days plus or minus 1 4 he would soon refer her for psychiatric treatment of an obsessional neurosis Some investigators have sought to avoid the inaccuracies of the hypothetical 28 day cycle by assuming that the postovulatory luteal phase (secretory phase) of the cycle is 14 days irrespective of the date of ovulation This too is susceptible of biologic variation particularly in relation to the life cycle of the corpus luteum

The most critical date in the menstrual cycle in regard to fertility is the date of ovulation If it is agreed that in a hypothetical 28 day cycle the mean date is the 14th day it is readily apparent from study of both temperature charts and datable pregnancies that ovulation can occur from the 3d to the 26th day and that the standard deviation is in the order of four days

1 Estrogenic Effects

Apart from variation in the range of physiologic limits of normative behavior of the endometrium to cyclic stimuli there is almost an infinity of possible abnormalities of response to stimuli which must be considered pathological If for example there is failure of ovulation the corpus luteum does not form and the luteal phase of the cycle is abolished However not infrequently there is endometrial bleeding at or near the expected date of menstruation this clearly is estrogen *withdrawal bleeding* a consequence of fall in estrogen level as FSH stimulation is diminished It is unfortunate that information regarding the hypophyseal cycle is not well *documented in human subjects* but it is a necessary pre-existing condition for the effective maintenance of the ovarian cycle It can safely be conjectured that many of the obscure disorders of the menstrual cycle may be traced to aberrations in the production of FSH and LH in respect to time and proportion but there is no definitive data which enables one to

equate pituitary changes with ovarian changes and subsequent endometrial changes except in the most exaggerated examples of pituitary disease such as Simmonds cachexia (pituitary necrosis) and pituitary neoplasms Another common disorder of the menstrual cycle is known variously as irregular maturation or irregular shedding Prolongation of the menstrual period and excessive flow are the characteristic symptoms of this disorder but the nonspecificity of these two symptoms is manifest The diagnosis can only be made with certainty on examination of adequately representative curettings in which the microscopic appearance of the endometrium exceeds the normal limits of variability In such cases it is not uncommon to see fragments of endometrium corresponding to the late proliferative phase other fragments compatible with the early progravid phase and still other fragments with an advanced predecidual response and the stigmata of leukocytic infiltration and disintegration characteristic of menstruation (Fig 7) Such endometria are undatable and can only suggest a lack of synchrony in the application of the successive stimuli of estrogens and progesterone If such lack of synchrony prevails for any considerable period of time the receptivity of certain areas in the end organ may be so modified that regularity is difficult to re establish



FIG 7 UNEVEN MATURATION AND IRREGULAR SHEDDING OF THE ENDOMETRIUM A focus of persistent secretory activity is seen in a predominantly proliferative endometrium × 45

The most frequently encountered abnormality of the endometrium which can be construed as an endocrine response is hyperplasia While advanced stages of this lesion are readily recognizable the presence or absence of lesser degrees is not readily calculable The minimal criteria for the diagnosis vary considerably from one pathologist to another In certain instances any curettings which deviate from the norm and are not truly neoplastic are lumped into a general wastebasket of "hyperplasia while in other instances minute descriptive and qualifying adjectives are used to classify the anatomic pattern Such variants as Swiss cheese hyperplasia polypoid or pseudopolypoid hyperplasia adenomatous hyperplasia combined glandular and stromal hyperplasia etc are all familiar and less common histopathologic changes such as hyperplasia with tubal metaplasia with squamous metaplasia with syncytial metaplasia are also found In all probability the specific morphologic variant is of little importance The significant common denominator is that endometrial hyperplasia represents either an exaggerated response to a normal amount of estrogen unopposed by adequate progesterone or to an abnormally high amount of elaborated estrogen Endometrial hyperplasia is a common cause of uterine bleeding either before during or after the menopause Pathologic examination of the ovaries associated with such endometria reveals almost any functional ovarian lesion one can name ranging from normal ovaries through cortico stromal hyperplasia to thecomatosis or even granulosa cell or theca cell tumors Statistically it is more common for the ovaries not to exhibit a distinctive lesion in such cases (Fig 8)

The clinical importance of endometrial hyperplasia once the pathologic diagnosis has been established unequivocally is readily apparent It is unwise to treat such patients with repeated small doses of estrogens However in some instances large doses of estrogens have been used for a short period to inhibit FSH production when estrogen is withdrawn bleeding ensues and on occasion a return to normal cyclic response has been noted Only rarely have such instances been documented by subsequent examination of endometrial biopsies to see if the abnormal anatomic pattern has been modified Likewise a hyperplastic endometrium is not very likely to be suitable soil for implantation correction of the anatomic pattern if possible is a prerequisite to successful infertility therapy when the lesion occurs in women of the childbearing age It is not unheard of for infertile patients with hyperplastic endometria to



FIG 8 SWISS CHEESE HYPERPLASIA OF THE ENDOMETRIUM The patient had been taking doses of stilbestrol up to 100 mg weekly (without medical advice) for 3 years There was no suggestion of atypical cellular morphology

give the impression of having cyclical menstruation even though the bleeding is merely due to temporary estrogen recession and is actually from focal necroses in the hyperplastic tissue Administration of progesterone during the latter half of the cycle has been used with some success to counterbalance the excess unopposed estrogen It is a notable pathologic observation that endometrial hyperplasia particularly at or near the menopause though a proliferative response to excess estrogen is not accompanied by any great degree of mitotic activity The most satisfactory explanation of this observation is that the hyperplasia develops gradually over successive menstrual cycles never returning quite to normal after each cycle because of the persistence of a relative estrogen excess

There is a considerable body of literature dealing with the relationship of endometrial hyperplasia and excessive estrogen stimulation to the subsequent development of adenocarcinoma of the endometrium While it is clear that in many cases hyperplasia and hyperestrogenism precede the development of carcinoma the mechanism by which the disturbance in function and the disturbance in structure produce neoplasia is not clear Nor do the most ebullient advocates of these theories insist that every case of endometrial cancer is related to these phenomena It is clear from the studies of Speert[37] that many cases of endometrial carcinoma evolve slowly progressing through stages of hyperplasia hyperplasia with cellular atypism on to frank adenocarcinoma To what extent this is related to increased estrogen activity is not clear However the problem can be brought into sharper focus when attention is paid to those adenocarcinomas of the endometrium which arise in younger women under the age of 40 a high percentage of such patients has a prolonged history of menstrual abnormalities compatible with hyperestrogenism and in many instances re examination of tissue obtained at curettage indicated the presence of the features of cellular atypism which suggest the possibility of such a sequence Gusberg[14, 15] has described a pattern of adenomatous hyperplasia which bears a significant relation to estrogenic lesions such as those produced by prolonged estrogen administration and granulosa cell tumors as well as with patients with recurrent episodes of functional bleeding he construes this pattern as a precursor to the development of corpus carcinoma Hertig and his associates [3] have described similar patterns as carcinoma *in situ* thereby indicating their belief that the lesions they describe are actually neoplastic at the time examined More conservative terminology has been used by Novak[44] and by Novak and Rutledge [3] they consider such lesions as atypical hyperplasia but note that it is not infrequently *followed* by the development of adenocarcinoma Particular attention has been paid to hyperplasia followed by carcinoma in postmenopausal women some observers feel that the unopposed action of estrogens from incompletely involuted ovaries can furnish sufficient stimulation of the endometrium to produce hyperplasia and then carcinoma (Figs 9 12) Smith and subsequently Woll *et al*[67] found hyperplasia of the ovarian cortical stroma and stigmata of theca cell overactivity to be more frequent in women with endometrial carcinoma than those without However the histologic point of reference for what con

stitutes cortico-stromal hyperplasia is not clearly defined Likewise the association of hyperplasia and adenocarcinoma of the endometrium with estrogen producing ovarian tumors has been cited to support the role of estrogens in endometrial carcinogenesis Unfortunately the occurrence of this association ranges from 3 3 per cent to almost 33 per cent depending upon which series of cases one reads One must perforce interpret such data as indicating the operation of factors in selection of material which weight the results *praeter naturam* The positive contribution of the various investigations into the possible role of estrogens in the development of endometrial cancer is that one can safely say that the morphologic pattern of adenocarcinoma on occasion evolves through stages which are best labeled atypical hyperplasia and that in some patients there is an associated hyperestrogenism This supports the dictum of the late James Ewing that a lesion need not be cancer or not cancer it may be on its way to becoming cancer It is likewise clear that if estrogens play any significant role in carcinogenesis the status of the end organ is also of significance be it pre intra or postmenopausal Certainly the development of cancer in any organ is a complex chemical and physical process and it is doubtful if any one substance acting alone can be implicated as an etiologic agent in the same sense that the pneumococcus is the cause of lobar pneumonia

Recently McKay *et al*[39] have shown that the histochemical constitution of the neoplastic endometrium resembles that of the progestational phase of the cycle in sharp contrast to hyperplastic endometria which resemble the estrogenic phase histochemically If the metabolism of neoplastic cells can be construed to reflect the mechanism of pathogenesis it is evident that estrogen stimulation alone is not a sufficient etiologic factor but that both estrogens and progesterone may be involved in the complicated sequence of chemical events related to the development of endometrial cancer

In the light of the discussion above it is easy to understand that there may be a considerable range of pathologic opinion when it comes to evaluating the curettings from an endometrium suspected of harboring an early adenocarcinoma Many observers are reluctant to classify a lesion as malignant unless there is incontrovertible morphologic evidence of irreversible cellular changes One of the inherent limits of the methods of morbid anatomy is that one must perforce interrupt a biologic process at a given instant of time to procure material for pathologic study Such study represents an attempt to extrapolate backwards to what is thought to have been the normal state of the tissue and to extrapolate forwards and predict the future course of the lesion Even as in carcinoma *in situ* of the cervix the evalu

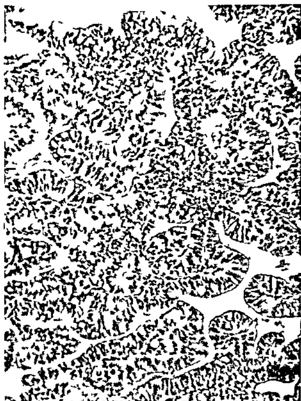


FIG 9 ATYPICAL ENDOMETRIAL HYPERPLASIA Many consultants considered this lesion to be adenocarcinoma but hysterectomy 3 years later failed to disclose even this degree of atypism × 125



FIG 10 ADENOCARCINOMA ARISING IN A HYPERPLASTIC ENDOMETRIUM (From a drawing in the collection of the late Dr Robert Meyer)



FIG 11 ADENOCARCINOMA OF THE ENDOMETRIUM WITH SECRETORY ACTIVITY Although neoplastic the cells are sufficiently well specialized to respond to endocrine stimuli The patient was premenopausal and a corpus luteum was present in one ovary × 100



FIG 12 SAME AS FIG 11 stained with periodic acid and digested with diastase to show glycopolysaccharide as one of the materials in the secretion × 100

ation of atypical endometria is limited by the fact that a diagnosis of malignancy must be made in the absence of the principal morphologic and behavioral feature of malignancy namely invasion While there can be no cavil at an attempt to diagnose carcinoma in its earliest (and most treatable) stages it must be realized that accuracy of prediction will be reduced when less distinctive criteria are employed There can be many loci on the morphologic spectrum between innocuous hyperplasia and obvious carcinoma The lack of unanimity of opinion as to when a given lesion is or is not carcinoma reflects this The basic premise of a pathologist s prediction that a given lesion will inevitably progress to invasion

metastasis and death if not treated i e is irreversible is based entirely upon morphologic congruence of the given lesion with lesions which have been known to have followed this course In view of the wide range of morphologic appearances which atypical endometria may exhibit the value of such predictions is reduced frequently the decision for hysterectomy must be based on clinical grounds such as the patient s age parity socio-economic status and her emotional attitude toward her disorder

The problem of diagnosis comes into sharp focus when curettings from a young woman exhibit borderline changes Not infrequently opinion may be divided regarding the nature of the lesion The comments by Grady[1] in interpreting one such controversial case merit quotation There is no question that an endometrial lesion like this in a young woman stands a very good chance of evolving into frank adenocarcinoma but it doesn t inevitably do so and the borderline between cases of severe adenomatous hyperplasia and frank adenocarcinoma is a very dim one indeed The situation is well borne out by the divergences in the opinion which are almost evenly divided between malignant and non malignant Given a lesion like this in a post menopausal woman or one who is around the time of the menopause I think that discretion is the better part of valor and hysterectomy is a perfectly justifiable procedure In a young woman I would like to be more conservative and examine more material (from repeated curettages) before an unequivocal diagnosis of adenocarcinoma is made In the light of this temperate statement the lack of unanimity of histopathologic criteria and the limited value of pathologic diagnosis in such cases as the motive and the cue to action become apparent Perhaps one reason for this lies in the fact that most of our thinking has been shaped by retrospective studies i e microscopic examination of previous curettings from women who have developed frank adenocarcinoma at varying intervals subsequently Only infrequently does the pathologist have the opportunity to study material from a lesion which has flourished and then regressed This points the need for prospective studies and the refinement of histopathologic criteria

Greene and his associates have shown that a hyperplastic endometrium is rarely found in post menopausal women They have also demonstrated that adenocarcinoma only infrequently arises in an endometrium with pre existing hyperplasia

Larson has written a critical review of the relationship of estrogens to endometrial cancer His conclusion is that there is but meager evidence to implicate estrogens in the etiology of endometrial carcinoma He points out that although carcinoma has developed in women who were being treated with estrogens this may well be coincidence for many postmenopausal women are given estrogens at an age when the incidence of endometrial carcinoma is maximal He points out that the lowest rate of association of endometrial carcinoma with feminizing ovarian tumors was found by Emge[10] whose studied population more closely reflects a random selection of cases than other reported series He points out that the concept of delayed menopause i e that women with endometrial carcinoma have a later onset of menopause than normal women because of persistent estrogen activity was based on inaccurate statistical data his own data indicate that menopause occurred in patients with carcinoma at 49 1 years and in the control group at 48 4 years He points out that endometrial cancer can develop in castrates His principal point of attack—one that embraces almost all studies in this field—is that of unconscious selection of cases and data inherent in the nature of the hospital or clinic population

If we attempt to reconcile Larson s attitude of not proven with the enthusiasm of other observers we must pay some attention to possible mechanisms of carcinogenesis Even as the cigarette has been implicated in the etiology of carcinoma of the lung by some others have steadfastly maintained that it has not been established that the cigarette is the criminal—it may merely have been present at the scene of the crime Berenblum[3] has shown that carcinogenesis may be a two stage process involving both initiating and promoting factors When he followed a single application of a known carcinogenic stimulus with continued applications of a nonspecific noncarcinogenic irritant he was able to produce carcinoma The role of estrogen in endometrial cancer may be similar It is not improbable that continued unopposed estrogen stimulation may serve to promote the development of carcinoma in the endometrium of a woman which has had the appropriate initiating stimulus which renders the cell susceptible of responding by neoplastic growth to estrogen promotion However this is conjectural The nature of the hypothetical initiating stimulus for the human endometrium is not known However a theory along these lines might serve to explain why after considerable estrogen therapy most women show merely hyperplasia while a few can develop cancer It might also explain why some women with atypical endometrial patterns which suggest possible neoplastic sequelae undergo regression while others progress to carcinoma Some observers have advanced the idea that endometrial carcinoma is merely one possible mode of expression for a pluriglandular disease citing its association with obesity diabetes mellitus proliferating and neoplastic lesions of the breast and the presence of primary carcinomas in other locations before and/or after the recognition

of the endometrial neoplasm While this is still merely a hypothesis and needs to be documented by careful statistical study of a truly random population it is compatible with the notion of estrogen as a cancer promoting substance which may act on a variety of end organs in concert with other disturbed endocrine equilibria

The preceding discussion has dealt with examples of gynecologic pathology in which there is some reason however tenuous to believe that we have achieved some insight into the nature of the disturbances However our ignorance is still larger than our knowledge and there remains the numerically significant number of cases in which after thorough gynecologic and pathologic examination we are forced to make a diagnosis of functional bleeding or dysfunctional flowing It is of some interest that the older term metropathia hemorrhagica implicated the uterus as the site of the disease Actually this is probably not so Meaker s maxim that functional bleeding represents the attempt of a perfectly normal hard working self respecting uterus to obey the dictates of a pair of crazy ovaries has much to recommend it Unfortunately our knowledge of what is going on in the ovaries of such patients is nil and it is only rarely that we even consider the role of the pituitary in determining the ovarian cycle One datum appears certain in these cases examination of excised uteri has consistently failed to reveal changes of any importance such uteri are monotonously normal The gap between endocrine theory and gynecologic practice has not been appreciably narrowed in this syndrome

Bleeding is the most striking clinical phenomenon in these patients and the bleeding may be severe recurrent and dangerous to the patient s general health A state of chronic anemia with acute exacerbations is not uncommon When medical management fails to restore such women to reasonable health hysterectomy must be resorted to merely to stop the continual blood loss While the majority of the patients may be over 30 years old a considerable proportion of them are younger and it is tragic that their reproductive capacity should be ablated However the inherent fertility of this group is low and it is fortunate that many of them do not develop functional bleeding until after one or more gestations

A typical example of functional bleeding in a 32 year old woman can be found as Cabot Case #34042 [5] Hysterectomy was performed and an anatomically normal uterus removed Although no cause for the bleeding was found there can be no doubt that in this patient hysterectomy was obligatory and the patient benefited thereby Meigs s comment there are many patients who come in with unexplained bleeding why they bleed I do not know is as much as anyone can safely say Mallory s comment It is well worth while occasionally to remind ourselves that the most careful histologic examination may reveal nothing in the face of severe functional disorders is a caution that most pathologists will hear with pleasure

2 Progestational Effects

The characteristic tissue response to progesterone stimulation is the formation of decidua This reaction is an inherent property of any adult stromal tissue derived from the mesenchyme of the urogenital ridge Even in the classical demonstration by Loeb preliminary sensitization of the stromal cells by estrogen is necessary Under most clinical circumstances there is sufficient circulating estrogen for this to occur Under physiologic conditions decidua is in William Hunter s words an efflorescence of the internal coat of the uterus and is shed as often as a woman bears a child or suffers a miscarriage The difference between the true decidua of pregnancy and the predecidua of the luteal phase of the menstrual cycle is one of quantity and not of kind Decidual transformation of the endometrial stroma is also seen in cases of extra uterine choriocarcinoma a response to the progesterone elaborated by the trophoblast Ectopic decidua has been described in pregnancy in many diverse locations notably the stroma of the endocervix and endosalpinx just beneath the peritoneal surface of the ovary under the serosa of the bladder and other loci in the pelvic peritoneum the appendix omentum sigmoid in the stroma of pelvic lymph nodes and even in the renal pelvis The observation by Weller that the distribution of the ectopic decidual response corresponds topographically to that of endometriosis lends support to theories of coelomic metaplasia in the genesis of this disease During pregnancy it is not uncommon to see decidual transformation of endometriomata in such locations as the ovary a previous laparotomy scar the umbilicus the lung etc Ectopic decidua can also be found in the absence of pregnancy particularly in the ovary after some injury to the corpus luteum has liberated progesterone which may exert a purely local effect

Decidual transformation is considered adaptive in that the swollen polyhedral cells contain abundant amounts of glycogen lipid and ribonucleoprotein which bear a nutritive relationship to the implanted blastocyst Precisely how long this function is maintained is difficult to state Certainly after placental angiogenesis has established a transport system between fetal and maternal circulation it can serve no useful function in that regard *However* the decidual efflorescence continues to increase in size and extent certainly until the uterine cavity is filled by

the growing embryo and its membranes It is only after the uterus is stretched by the continued growth of its contents that the decidua becomes attenuated As gestation continues both estrogens and progestational substances are elaborated in the placenta presumably by the syncytiotrophoblast Extremely high levels of manufacture can be achieved as evidenced by studies of pregnanediol excretion during pregnancy It is not surprising therefore that the large amounts of progesterone are above the threshold for deciduoma response and that ectopic decidua can form so readily and so diffusely during the last two trimesters Likewise it is not surprising on occasion to find decidual transformation in the endometrium of a newborn infant as well as secretory vacuolation Pregnanediol has been isolated from the urine of newborn male infants but whether it derives from the maternal tissues the placenta or the fetal adrenal cortex remains undetermined

Not every stromal cell in the endometrium is converted to a decidual cell Hamperl[16] and Hellweg[18 19] have described endometrial granulocytes (*Kornchenzellen*) formerly thought to be lymphocytes which do not respond by decidual transformation The intracytoplasmic granules are stained by *Weigert s fibrin stain as well as other methods* These cells increase markedly in number during the luteal phase of the cycle and are even more prominent during early gestation They are absent in the stroma of hyperplastic endometria It is clear that they respond in some way to progesterone stimulation but their significance is not understood I have observed such cells in neonatal endometria with decidual transformation and they are as distinct from the decidual cells in the infant as in the adult

The importance of the decidual reaction in relation to fertility is that it presumably reflects the events in the corpus luteum An adequate endometrial biopsy taken at or just before the onset of flow usually furnishes evidence regarding the adequacy of the predecidual response In this situation a false positive is rare for adequate predecidua is rarely obtained unless there is a well formed functioning corpus luteum but a negative observation can on occasion be misleading for effective predecidual response may be inhibited either by excess circulating estrogens or by failure of the end organ to react due to temporary local factors On occasion an endometrial biopsy will show a true decidual response in a progestational endometrium of early pregnancy Such biopsies should be examined in multiple sections to identify trophoblastic elements If none is found it is possible that the early implanted ovum has not been disturbed Many such cases have carried to term without difficulty

After abortion or miscarriage decidua degenerates and is sloughed in the lochia If decidua is retained it undergoes degeneration and becomes hyalinized If for any reason there is delayed postabortal or postpartum bleeding curettage is in order and examination of the curettements will often reveal the hyalinized decidua along with cellular debris and an inflammatory exudate When decidua persists in the walls of endometrial spiral arterioles and venules the hyaline change stiffens the structures and favors vascular ectasia and the development of subinvolution of the placental site and further bleeding

An infrequently encountered syndrome is that of a persistent corpus luteum in which the corpus luteum becomes cystic and does not involute It is characterized by irregular bleeding from an endometrium which shows a combination of regenerative changes menstrual like dissolution and a persistent decidual reaction It is analogous to pseudopregnancy in animals in that it represents progesterone predominance with an undercurrent of rising estrogen stimulation It is one of the few instances in which a clinical disease entity is due to excess progesterone

In relation to pregnancy progesterone appears to be necessary for a time to maintain the integrity of the implanted ovum and oophorectomy during the first two months may initiate abortion However *after the eighth gestational week the corpus luteum* of pregnancy is of less significance for progesterone production than the syncytiotrophoblast By the same token there is no substantive evidence that administration of corpus luteum extract of progesterone has any beneficial effect in cases of threatened abortion Symeonidis[60] has produced an eclampsia like syndrome in rats by injecting massive doses of progesterone during their last trimester hypertension albuminuria and edema were observed Premature placental separation and intra uterine fetal death were frequent The changes in the maternal liver and kidneys were comparable to changes seen in some cases of human eclampsia However there is no evidence to implicate hyperprogesteronism in human eclampsia in general as toxemia of pregnancy progresses both estrogen and progesterone levels diminish presumably due to degenerative changes in the syncytiotrophoblast

B Myometrium

At birth the corpus uteri is relatively short being about half as long as the cervical portion As growth progresses the corpus increases in size until it is about three times as long and three times as wide as the cervix While much of the growth stimulus is nonspecific estrogens are necessary for proper development and an infantile or juvenile uterus is characteristic of hypoestrogenic states

During pregnancy there is both hyperplasia and

hypertrophy of the smooth muscle fibers which compose the uterine wall and these changes recede after delivery part of the process of normal involution. The actual changes in fiber size are enormous for this relatively short period individual fibers can become as much as seven times wider and eleven times longer than normal. The mechanical force of stretching to accommodate the enclosed fetus amniotic fluid and membranes plays a major role in this growth process but there is an increase in weight from 30 gm to about 1000 gm and it represents a true growth process. To what extent steroids and gonadotropins enhance the mechanical effects cannot be determined but there is little reason to doubt their importance.

The uterine atrophy which is common in elderly postmenopausal women is accompanied by a true loss of muscle substance and an apparent increase in fibrous interstitial tissue. This is secondary to estrogen failure.

The characteristic lesion of the myometrium is the leiomyoma a simple expanded overgrowth of relatively mature smooth muscle cells. The evidence that such benign neoplasia is in part related to estrogenic stimuli is their appearance during the period of adult sexual life and their involution and regression after the menopause. Like the normal myometrium they increase in size during pregnancy and often undergo degenerative changes probably secondary to circulatory inadequacy within the tumor. Less frequently observed is a peculiar atypical hyperplasia of leiomyomas during pregnancy in some instances the cellular atypism has some of the features of leiomyosarcoma but close examination soon dissipates this misleading appearance.

Lipschutz [8] has produced fibroid tumors in guinea pigs by administration of estrogens. This has given rise to the mistaken idea that leiomyomas may be endocrine dependent tumors. However Nadel [9] has shown that such fibroids are not derived from smooth muscle but are actually desmoid like tumors arising from fascia and connective tissue. No relationship to the usual human leiomyoma can be construed.

Tannhauser [61] has described rejuvenation of the myometrium in cases of adenocarcinoma of the endometrium in postmenopausal women. He points out that in many such cases the myometrium is not atrophic but resembles the myometrium of a woman in the prime of life. The changes are considered to be either failure of involution or reactivation. Similar changes were found in leiomyomas in these uteri. He ascribes this to the persistent high level of estrogens found in many women who have endometrial cancer.

IV FALLOPIAN TUBES

In spite of the fact that fertilization takes place in the fallopian tubes they are probably the portion of the female genital tract least affected by hormones particularly insofar as clinical effects are concerned. The endosalpingeal epithelium does respond cyclically to ovarian hormones. During the estrogenic phase the epithelium is tall with broad ciliated cells and narrow intercalated cells. During the luteal phase the ciliated cells become lower and a ragged appearance is given to the surface by the protrusion of secretory cells. These changes are accentuated during menstruation and gestation the epithelium being almost flat in many zones. In cases of endometrial hyperplasia there may be an associated hyperplasia of the tubal epithelium a response to estrogenic stimuli. The endosalpingeal stroma regularly undergoes focal decidual transformation during pregnancy. This list of minor morphologic alterations is only to be expected the endosalpinx like the endometrium and the endocervix is developed from the Mullerian apparatus and is responsive as an end organ to the same stimuli lesser in degree than the endometrium comparable in degree to the endocervix but essentially the same in kind.

In ectopic gestations 95 per cent of which occur in the tube a true implantation site forms. There is a patchy irregular decidual reaction in the endosalpinx and its relation to trophoblast is the same as in the endometrium. The other changes in the tubes associated with ectopic pregnancy are nonendocrine being chiefly mechanical in nature. During ectopic gestation the endometrium may or may not respond to the usual endocrine stimuli. Romney and his associates [5] studied the endometrium in 115 cases of ectopic pregnancy finding that in 30 per cent of the cases the endometrium was in the proliferative phase in 40 per cent in the secretory phase in 10 per cent in the menstrual or regenerative phase and only 20 per cent showed true decidua formation. The limited value of endometrial biopsy in suggesting a diagnosis of ectopic pregnancy is evident from these data (Fig 13).

A nonendocrinologic lesion of the tube which merits attention at this point is the so called lipogranuloma secondary to examinations for tubal patency in fertility studies by radiologic techniques. If an oil based contrast medium is employed it may initiate a chronic granulomatous inflammatory reaction which will effectively close the tube. The extent of the reaction is variable. Occasionally one may encounter a small nidus of multinucleated cells epithelioid cells and lipophages on the surface of the ovary or at the fimbriated end of the tube many months or even years after such an instillation in

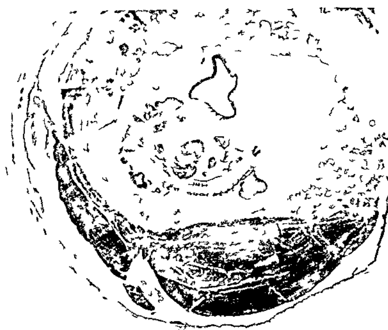


FIG 13 ECTOPIC PREGNANCY FALLOPIAN TUBE The retro placental hematoma is typical The chorionic villi are numerous and the trophoblast well devel ped despite the abnormal location × 7 (Specimen furnished by Dr Margaret R Loeffler)

such instances the lesion is purely focal and of no clinical significance However in an occasional case the reaction may be severe and extensive involving the entire tube Whether or not there is an increased susceptibility for previously inflamed tubes to develop this reaction is not certain However the problem can be obviated by the use of a contrast medium which is water soluble (Fig 14)

V OVARY

There are four principal lacunae in our information which prevent an effective correlation of structure with function in regard to ovarian lesions First there is no easily applicable method for localizing a given hormone to a given cell The usual fat stains indicate the presence of neutral fat not steroid hormones The latter are only demonstrable by histochemical techniques and the application of these techniques requires fresh tissue and elaborate facilities Even utilizing histochemical techniques a battery of special methods must be used to characterize the substances identified and the techniques are not sufficiently refined to discriminate precisely among certain specific members of classes of steroid hormones The bulk of our information to date has come from clinical observations bioassays and chemical extractions Second there is no unanimously accepted theory of the embryogenetic differentiation of certain significant structures in the ovary Although theca interna theca externa and cortical stroma are mesenchymal in origin recent investigations indicate that the granulosa cells may possibly be of sex cord origin There is not a uniform correlation between the embryogenesis of a cell type and the hormone it may produce under conditions of hyperplasia or neoplasia Third it has not been possible to clarify the relationship among pituitary adrenal and ovarian hormones to the extent that one can safely classify adreno genital syndromes on a rational basis We are still at the stage of empirical evaluation of sporadic cases In large measure this failure is due to the difficulty in determining which hormones are significant in a given case where they are elaborated and what effects they have on other hormonal functions in the given patient either as the syndrome developed or at the time of study Fourth although the pituitary ovarian relationship has been worked out with some care in experimental animals we still lack precise quantifiable data regarding its relationship in humans In the human female we are able to titrate FSH against estrogens and LH against progestational substances only in the most qualitative way and our knowledge of the interrelation between FSH and LH is indeed primitive

It becomes evident that the methods of normal and morbid anatomy have conspicuously failed to solve the problems of ovarian structure in relation to function and we must rely in large measure for support from allied disciplines such as experimental embry



FIG 14 LIPOGRANULOMA FALLOPIAN TUBE FOLLOWING THE INSTILLATION OF AN OIL BASED RADIO-OPAQUE MEDIUM FOR DEMONSTRATION OF TUBAL PATENCY There is a diffuse granulomatous inflammatory reaction with numerous systems of giant cells some of which contain asteroid bodies $\times$ 48 (*Inset* $\times$ 125)

ology biochemistry and endocrinology However lest we dismiss too eagerly the value of anatomic studies both normal and pathologic as mere morphology we must realize that without the elements of structure these allied disciplines would have no substantive frame of reference for their contributions

1 The Graafian Follicle and the Corpus Luteum

At birth the ovary contains a full complement of germ cells (ova) closely packed and separated into individual compartments by an inconspicuous framework of sustentacular cells A few follicles may have been stimulated by maternal hormones to undergo expansion proliferation of granulosa cells and partial luteinization of theca interna Such evidence of follicle maturation persists through childhood indicating the possibility of a self regulating mechanism of the ovary It is not uncommon to observe atretic follicles in the ovaries of a newborn infant or in a child indicating that the cycle of follicle maturation and atresia can be completed at any time before or after birth (Fig 15) However follicle rupture with extrusion of an ovum (ovulation) does not occur until puberty Concurrently luteinization of granulosa cells does not occur until ovulation Female puberty is nothing more or less than the onset of cyclic ovulation and corpus luteum formation in response to cyclic stimulation from the pituitary An infrequent pediatric syndrome (Turner s triad) consists of ovarian agenesis dwarfism and pterygium Otherwise congenital failure of ovulation is essentially unknown However the hypoestrogenism associated with severe hypothyroidism (whether congenital as in cretins or acquired as in myxedema) may induce ovulatory failure also in Simmonds disease (usually post partum pituitary necrosis) there are hypoestrogenism and ovulatory failure due to failure of the pituitary to elaborate FSH The morphologic correlatives of these diseases are clear in Turner s syndrome only rudimentary vestiges of ovarian tissue are found in severe hypothyroid and hypopituitary states the ovary is involuted prematurely if the onset of the generalized endocrine disorder occurs before menopause

The endocrine functions of the ovary do not reside in the germ cells Until fertilized they are merely passive structures and the endocrine activity which surrounds them has no appreciable effect upon the germ cells themselves Germ cells are not even indigenous to the ovary they are not derived from germinal epithelium (better labeled ovarian peritoneum or mesothelium) Germ cells develop in the entoderm along the mid line and migrate into the ovary as it is being formed Tumors and anomalies of germ cells dysgerminomas and teratomas are not endocrine tumors except insofar as their replacement of functioning ovarian tissue may ablate ovarian function and defeminize a given patient The only

exception to this is in an occasional instance of so called struma ovarii (adult teratoma with predominance of thyroid tissue) which may respond to the same stimulus as the normal thyroid in the development of hyperthyroidism One case in my own experience stands out A woman of 35 developed classical Graves disease was treated with iodine and thiourea derivatives then by subtotal thyroidectomy She improved for a few months then her symptoms recurred There was no evidence of recurrent goiter radioactive iodine was given and detected in large quantities in an adnexal mass which had not previously been palpable At laparotomy cystic ovarian tumor was removed Microscopic examination showed typical struma ovarii with severe diffuse hyperplasia of the thyroid tissue However struma ovarii is uncommon enough and the addition of hyperthyroidism to it is so rare and bizarre that further analysis is supererogatory In general germ cell tumors and germ cell malformations are inert However choriocarcinomatous elements may occur in a teratocarcinoma and produce gonadotropic effects

The graafian follicle when fully developed just prior to ovulation contains the ovum which is surrounded by granulosa cells These are sharply delimited from the theca interna (Fig 16) After ovulation both granulosa cells and theca interna participate in the formation of the corpus luteum Theca interna is readily distinguishable topographically from theca externa but the latter layer blends imperceptibly into the stroma of the ovarian cortex and does not participate in corpus luteum formation The term corpus luteum merely means yellow body and is a literal translation of the French term *corps jaune* This refers to the yellow color of the scalloped border of plump polyhedral cells at the periphery It is noteworthy that the yellow color becomes more intense during the latter half of the life of the corpus luteum and corresponds with the amount of neutral fat present not with the hormone elaborated Corner Hartman and Bartelmez[7] have studied in detail the life cycle of the corpus luteum in the rhesus monkey Clinical and pathologic observations indicate that a similar life cycle is present in the human female In essence the process of luteinization can be subdivided into four distinct but sequential stages proliferation, vascularization maturation, and retrogression During the stage of maturation, large amounts of progesterone are synthesized within the luteinized cells during the stage of retrogression the progesterone is liberated into the blood stream and acts upon the endometrial stroma producing a predecidual reaction On the average it takes nine days for the corpus luteum to reach its maximum of hormone synthesis the steroid is released over the next five days If pregnancy does not supervene the stage of retrogression continues and the corpus luteum involutes to become a corpus albicans a scalloped hyaline mass during the next few cycles During this period of retrogression it is bright yellow and rich in neutral fat but poor in hormones If pregnancy ensues a corpus luteum of pregnancy is formed This organoid structure has a somewhat longer life cycle Progesterone is elaborated in in

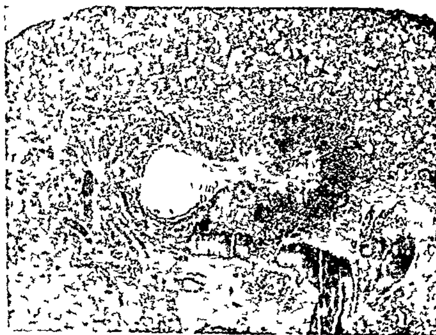


FIG 15 OVARY OF NEWBORN SHOWING ATRETIC FOLLICLE × 62



FIG 16 LINING OF GRAAFIAN FOLLICLE showing a sharp line of demarcation between granulosa cells and theca interna × 137

creasing quantities under stimulation from chorionic gonadotropins for a period of six to twelve weeks However after the eighth or tenth week of gestation progesterone synthesis in the corpus luteum of pregnancy diminishes as the elaboration of progesterone in syncytiotrophoblast increases The corpus luteum of pregnancy is not necessary for the maintenance of an implanted ovum certainly after the first trimester and probably after the end of the second month of gestation in most cases This information becomes of clinical importance in planning the date for laparotomy for removal of an adnexal mass discovered on examination early in pregnancy

The most prominent cell in the corpus luteum is the transformed granulosa cell The normal granulosa cell is a small cuboidal cell with a round nondescript nucleus and an inconspicuous amount of pallid cytoplasm Under the influence of LH the cytoplasm becomes swollen abundant rich in fine vacuoles and brightly eosinophilic The analogy to decidual transformation of the naked nucleus type of endometrial cell is obvious Along with this cytoplasmic change in the granulosa cells the theca interna at the periphery of the granulosa becomes transformed albeit to a lesser degree these are the so called theca lutein or paralutein cells White *et al*[66] have indicated by histochemical techniques that the theca interna is the precursor of the so called K cells in the corpus luteum and that these are associated with the elaboration of progesterone and possibly keto steroids whereas the granulosa lutein cells elaborate progesterone They point out that when there is a failure of trophoblast i e a blighted ovum the corpus luteum undergoes early regression and that a striking feature of this regression is the colloid degeneration of the K cells It is not surprising therefore that the corpus luteum of pregnancy when seen at term or at any time during the last trimester should be a degenerating organ rich in neutral fat and colloid material but almost devoid of hormone The ectopic decidual reaction in the ovary during pregnancy has already been discussed it is almost uniformly present to some degree and is evidence that progesterone or progesterone like substances stimulated an estrogen sensitized cell which had the inherent capacity to react (Figs 17 20)

Although pathologic lesions of the corpus luteum are not rare they infrequently give rise to any disorder that can be called endocrine in nature The only exception is the persistent corpus luteum cyst which retains its progestational function and produces a disturbance in the menstrual cycle and in the endometrium as discussed above Such lesions as the corpus luteum hemorrhagicum or corpus luteum hematoma produce signs and symptoms of an acute abdomino pelvic emergency Shaw's[5] statement that the corpus luteum is by nature a cystic organ is as valid today as it was 30 years ago and his description of cysts tarry cysts and hematomas has not been rivaled In only one respect can one take issue with

this summary of the classical view The term luteoma has fallen into disrepute It is evident that ovarian tumors composed of polyhedral cells with eosinophilic sudanophilic cytoplasm represent luteinized granulosa cell tumors The transitory life of the corpus luteum and the fact that the luteinized cells are in reality either granulosa or theca has rendered the term obsolete

It is evident that ovulation and the formation of an adequate corpus luteum are necessary prerequisites for human fertility In practice we determine these occurrences by endometrial biopsies In theory

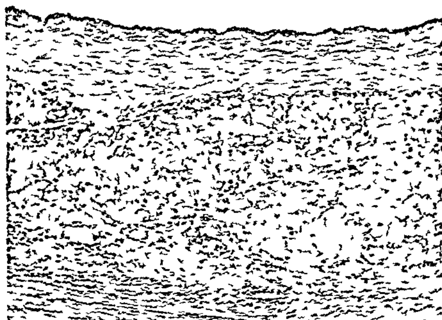


Fig 17 Corpus luteum cyst ovary showing luteinized granulosa cells in the wall × 125

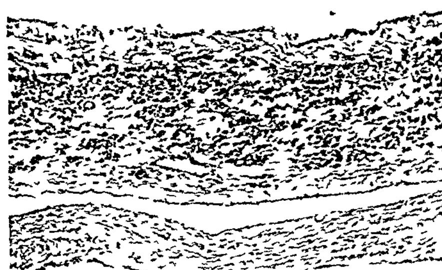


Fig 18 The same corpus luteum cyst as Figure 17 showing theca lutein cells in the wall × 125



FIG 19 ECTOPIC DECIDUAL REACTION ON THE SURFACE OF THE OVARY just below the peritoneal lining The patient was not pregnant but had an inflammatory lesion in the ovary which disrupted a nearby corpus luteum × 225

the appropriately synchronized cyclic phenomena of ovulation and luteinization depend upon the synchrony of FSH and LH elaboration by the pituitary one hormone after the other and in the correct proportion An FSH/LH ratio at a given instant of time must be necessary for ovulation to occur and there must be (within limits) a rate of increase of LH and a rate of decrease of FSH after ovulation as a condition for the formation of an anatomically normal corpus luteum which presumably will synthesize and elaborate the correct amount of progesterone in relation to the amount of estrogen for the appropriate changes to occur in the endometrium should a fertilized ovum be implanted It is regrettable that we are able to quantify such items in the bodily economy only qualitatively The reciprocal relation of FSH and LH is poorly understood yet it underlies many of the disorders of ovulation menstruation and fertility which we cannot deal with systematically at the present time

After the menopause ovulation and luteinization cease Presumably the menopause is *hypophyseal in origin and ovarian in expression* In any event the postmenopausal ovary has no more germ cells Most of them were lost by atresia but between 400 *and 500 were extruded and any number from zero* to 20 were fertilized There remains only the ovarian cortex Occasionally one can see a small remnant of granulosa cells forming a small clump in the ovarian cortex of a woman of 60 years of age or more Robert Meyer called these *granulosaballen* and thought that *granulosa tumors arose from them* this idea has not met with universal acceptance The ovarian cortex after the menopause has been the object of some interest Some observers have accumulated evidence to show that this cortex continues to elaborate estrogens in varying amounts and have attempted to equate continued estrogen production unopposed by progesterone production with the development of certain lesions notably endometrial carcinoma An ill defined lesion labeled corticostromal hyperplasia has been postulated This lesion is defined in terms of thickening scalloping and basophilia of the cortex However the lesion remains ill defined because there are no precise anatomic criteria which enable one to distinguish a hyperplastic cortex and a normal cortex either at a given age or at a given interval after the menopause In

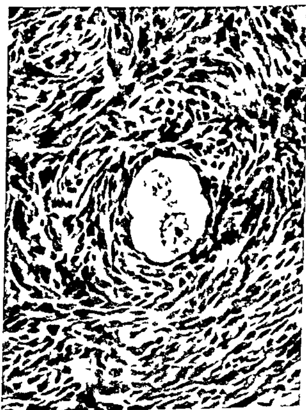


FIG 20 TWIN OVA This is one possible mechanism of twinning—provided both ova develop into a maturing follicle that both ova are extruded and viable at ovulation and that both are fertilized × 475

essence given a woman of 58 years 10 years post menopausal one cannot determine with any degree of precision how thick, how scalloped or how basophilic the cortex of her ovary should be While indubitably such tissues elaborate estrogens the proponents of these theories offer no evidence regarding the quantity of these estrogens or which specific estrogenic substances are elaborated It is only in the most strikingly abnormal cases that one can procure any unanimity of pathologic opinion that the anatomic changes present constitute an abnormality and even then there is no unanimity of opinion regarding cause and effect relationships nor any illumination cast upon the norm Such terms as estrogenic stroma while representing a legitimate hypothesis remain hypothetical

Not only is it impossible to state how much if any estrogen is being elaborated in the ovarian cortex of any given postmenopausal woman but the ability of the ovaries to interconvert the steroid hormones makes it difficult to impute with certainty a purely estrogenic function to this tissue Likewise the anatomic criteria for a diagnosis of cortico-stromal hyperplasia are not clear No one has yet defined just how much cortical stroma is normal for a postmenopausal womans ovary or what the limits are Possibly quantitative topographic studies might be of value in defining this further

Boddick and Greene find that there are no specific changes yet reported in the ovaries from women with endometrial carcinoma which cannot be found to an equal degree in those without carcinoma However the estrogenic function of cortical stroma has been studied by Scully in relation to ovarian neoplasms which are not usually considered functional He observed that in an occasional Brenner tumor the stroma is not indifferent and merely fibrous but is composed of plump spindle cells resembling theca Similar changes can be seen in an occasional Krukenberg tumor in which there is a traditionally sarcomatoid i e theca like proliferation of the stroma in association with the metastatic tumor In such cases it is not uncommon for there to be an associated endometrial hyperplasia and for the patient to present clinically with vaginal bleeding from the stimulated endometrium

2 Functional Ovarian Tumors

The classification of the so-called functional ovarian tumors has depended partly upon their clinical effects i e masculinization feminization defeminization etc upon the endocrine substances presumably elaborated i e estrogens androgens or both and upon their morphology No one of these three properties is completely satisfactory Many examples of these tumors are without clinical or hormonal evidence of functional activity We have learned that theca cells can elaborate androgens as well as estrogens In fact hormones are neither male nor female neither masculinizing nor feminizing This generalization is true for similar tumors in the male The clinical effects of the functional tumors vary with the time of the patients life the state of the end organs as well as with the specific substances elaborated In the presence of clinical and endocrinologic ambiguities it is wiser to classify this group of tumors on the basis of their morphology

Morris and Scully [65] have proposed such a classification Despite the uncertainties of our knowledge of the embryogenesis of certain cells within the ovary this classification has much to recommend it

- I Sex cord mesenchyme tumors
  - A Granulosa theca cell tumors and their luteinized forms
  - B Sertoli Leydig cell tumors (arrhenoblastomas)
  - C Sex cord mesenchyme tumors of uncertain or mixed cell types
- II Lipoid cell tumors (including tumors in which origin from adrenal hilar Leydig or lutein cell is uncertain)
- III Germinoma teratoma group (including choriocarcinoma struma ovarii)
- IV Mixed germinoma sex cord mesenchyme tumors (gonadoblastomas)
- V Miscellaneous tumors with functioning stroma

The choice of the term sex cord mesenchyme tumor instead of mesenchymoma reflects the uncertainty whether granulosa cells are truly mesenchymal in origin or whether they are derived from primitive sex cords The term Sertoli Leydig cell tumor reflects the analogy to tumors in the male gonad composed of both tubular elements and polyhedral cells of interstitial type One might cavil that the hilar cell tumors are best classified with the sex cord mesenchyme tumors rather than with the lipoid cell tumors particularly if one subscribes to the theory that the polyhedral cells in Sertoli Leydig cells are identical with the hilar Leydig cells

The granulosa cell and theca cell tumors are conveniently considered as a group Though pure forms of each cell type have been described they are most often mixed In general these tumors are estrogenic and feminizing when functional The granulosa cell tumor was first reported in 1859 by Rokitansky described histologically by von Kahlden in 1895 shown to be estrogen secreting by Schuschania in 1930 and correlated with clinical manifestations by Robert Meyer in 1931 The thecoma was first segregated from other spindle cell tumors of the ovary by Loeffler and Priesel in 1932 and its estrogenic functions appreciated by Traut and his colleagues a few



FIG 19 ECTOPIC DECIDUAL REACTION ON THE SURFACE OF THE OVARY just below the peritoneal lining The patient was not pregnant but had an inflammatory lesion in the ovary which disrupted a nearby corpus luteum × 225

the appropriately synchronized cyclic phenomena of ovulation and luteinization depend upon the synchrony of FSH and LH elaboration by the pituitary one hormone after the other and in the correct proportion An FSH/LH ratio at a given instant of time must be necessary for ovulation to occur and there must be (within limits) a rate of increase of LH and a rate of decrease of FSH after ovulation as a condition for the formation of an anatomically normal corpus luteum which presumably will synthesize and elaborate the correct amount of progesterone in relation to the amount of estrogen for the appropriate changes to occur in the endometrium should a fertilized ovum be implanted It is regrettable that we are able to quantify such items in the bodily economy only qualitatively The reciprocal relation of FSH and LH is poorly understood yet it underlies many of the disorders of ovulation menstruation and fertility which we cannot deal with systematically at the present time

After the menopause ovulation and luteinization cease Presumably the menopause is hypophyseal in origin and ovarian in expression In any event the postmenopausal ovary has no more germ cells Most of them were lost by atresia but between 400 and 500 were extruded and any number from zero to 20 were fertilized There remains only the ovarian cortex Occasionally one can see a small remnant of granulosa cells forming a small clump in the ovarian cortex of a woman of 60 years of age or more Robert Meyer called these granulosaballen and thought that granulosa tumors arose from them this idea has not met with universal acceptance The ovarian cortex after the menopause has been the object of some interest Some observers have accumulated evidence to show that this cortex continues to elaborate estrogens in varying amounts and have attempted to equate continued estrogen production unopposed by progesterone production with the development of certain lesions notably endometrial carcinoma An ill defined lesion labeled corticostromal hyperplasia has been postulated This lesion is defined in terms of thickening scalloping and *basophilia of the cortex However the lesion remains* ill defined because there are no precise anatomic criteria which enable one to distinguish a hyperplastic cortex and a normal cortex either at a given age or at a given interval after the menopause In

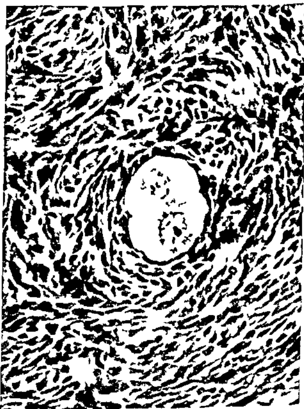


FIG 20 TWIN OVA This is one possible mechanism of twinning—provided both ova develop into a maturing follicle that both ova are extruded and viable at ovulation and that both are fertilized × 475

essence given a woman of 58 years 10 years post menopausal one cannot determine with any degree of precision how thick how scalloped or how baso philic the cortex of her ovary should be While in dubitably such tissues elaborate estrogens the pro ponents of these theories offer no evidence regarding the quantity of these estrogens or which specific estrogenic substances are elaborated It is only in the most strikingly abnormal cases that one can pro cure any unanimity of pathologic opinion that the anatomic changes present constitute in abnormality and even then there is no unanimity of opinion re garding cause and effect relationships nor any illu mination cast upon the norm Such terms as estro genic stroma while representing a legitimate hy pothesis remain hypothetical

Not only is it impossible to state how much if any estrogen is being elaborated in the ovarian cortex of any given postmenopausal woman but the ability of the ovaries to interconvert the steroid hormones makes it difficult to impute with certainty a purely estrogenic function to this tissue Likewise the ana tomic criteria for a diagnosis of cortico stromal hyper plasia are not clear No one has yet defined just how much cortical stroma is normal for a postmeno pausal woman s ovary or what the limits are Pos sibly quantitative topographic studies might be of value in defining this further

Roddick and Greene find that there are no specific changes yet reported in the ovaries from women with endometrial carcinoma which cannot be found to an equal degree in those without carcinoma How ever the estrogenic function of cortical stroma has been studied by Scully in relation to ovarian neo plasms which are not usually considered functional He observed that in an occasional Brenner tumor the stroma is not indifferent and merely fibrous but is composed of plump spindle cells resembling theca Similar changes can be seen in an occasional Kruken berg tumor in which there is a traditionally sarco matoid i e theca like proliferation of the stroma in association with the metastatic tumor In such cases it is not uncommon for there to be an associated endometrial hyperplasia and for the patient to present clinically with vaginal bleeding from the stimulated endometrium

2 Functional Ovarian Tumors

The classification of the so called functional ovarian tumors has depended partly upon their clinical effects i e masculinization feminization defemini zation etc upon the endocrine substances pre sumably elaborated i e estrogens androgens or both and upon their morphology No one of these three properties is completely satisfactory Many examples of these tumors are without clinical or hormonal evidence of functional activity We have learned that theca cells can elaborate androgens as well as estrogens In fact hormones are neither male nor female neither masculinizing nor femi nizing This generalization is true for similar tumors in the male The clinical effects of the functional tumors vary with the time of the patient s life the state of the end organs as well as with the specific substances elaborated In the presence of clinical and endocrinologic ambiguities it is wiser to classify this group of tumors on the basis of their morphology

Morris and Scully [8] have proposed such a classi fication Despite the uncertainties of our knowledge of the embryogenesis of certain cells within the ovary this classification has much to recommend it

- I Sex cord mesenchyme tumors
  - A Granulosa theca cell tumors and their lu teinized forms
  - B Sertoli Leydig cell tumors (arrhenoblas tomas)
  - C Sex cord mesenchyme tumors of uncertain *or mixed cell types*
- II Lipoid cell tumors (including tumors in which origin from adrenal hilar Leydig or lutein cell is uncertain)
- III Germinoma teratoma group (including cho riocarcinoma struma ovarii)
- IV Mixed germinoma sex cord mesenchyme tu mors (gonadoblastomas)
- V Miscellaneous tumors with functioning stroma

The choice of the term sex cord mesenchyme tumor instead of mesenchymoma reflects the uncer tainty whether granulosa cells are truly mesenchymal in origin or whether they are derived from primi tive sex cords The term Sertoli Leydig cell tumor reflects the analogy to tumors in the male gonad composed of both tubular elements and polyhedral cells of interstitial type One might cavil that the hilar cell tumors are best classified with the sex cord mesenchyme tumors rather than with the lipoid cell tumors particularly if one subscribes to the theory that the polyhedral cells in Sertoli Leydig cells are identical with the hilar Leydig cells

The granulosa cell and theca cell tumors are con veniently considered as a group Though pure forms of each cell type have been described they are most often mixed In general these tumors are estrogenic and feminizing when functional The granulosa cell tumor was first reported in 1859 by Rokitansky de scribed histologically by von Kahlden in 1895 shown to be estrogen secreting by Schuschiania in 1930 and correlated with clinical manifestations by Robert Meyer in 1931 The thecoma was first segregated from other spindle cell tumors of the ovary by Loeffler and Priesel in 1932 and its estrogenic func tions appreciated by Traut and his colleagues a few

years later McKay Robinson and Hertig[36 7] demonstrated by non specific histochemical methods that the estrogenic activity of granulosa theca tumors could be ascribed to the theca cell The pure varieties of microfollicular granulosa cell tumor are often inert but the pure theca cell tumors are often quite highly estrogenic

Microscopically granulosa cell tumors exhibit a wide variety of patterns—microfollicular trabecular pseudo tubular plexiform and diffuse (sarcomatoid) however these tend to overlap and be admixed within any one given specimen Often the apparent stroma of the granulosa cell tumor is in reality composed of small bundles of theca cells surrounding small blood vessels this effect can be demonstrated on occasion by a stain for lipoid in which the theca cells stain positively for intracytoplasmic neutral fat but the prominent epithelial granulosa cell aggregates are negative It must be emphasized that the stains for neutral fat which correlate well with the yellowness of the tumor on section do not stain steroid hormones and correlate poorly with functional manifestations The microscopic appearance of theca cell tumors typically is that of interlacing bundles of short plumpish spindle cells with a variably collagenized stroma On occasion these tumors blend imperceptibly with the inert ovarian fibroma *the best criteria for distinguishing a the*coma from a fibroma are the size and shape of cells the amount and distribution of collagen and the presence or absence of stainable neutral fat In general the fibroblasts have longer nuclei with more pointed ends stellate nuclei are found on occasion The cytoplasm of thecomas is more abundant and often shows fine foaminess under reduced illumination Diffuse abundant collagen is more characteristic of fibromas but occasional intercalated hyalinized plaques are found in thecomas Even using these criteria an occasional case will be difficult to evaluate

The histogenesis of these tumors has been debated at length Robert Meyer attributed the origin of granulosa cell tumors to *granulosaballen* nests of granulosa cells which remain in the ovarian cortex after menopause Robinson advanced the idea that they develop from adult graafian follicles Furth and Butterworth thought they arose from disorganized follicles McKay *et al*[33] recently advanced the idea that they arose from atretic follicles McKay also indicated that theca cell tumors might arise in hyperplastic ovarian cortical stroma citing the observation that in many cases the contralateral ovary exhibited such a phenomenon Although Traut and Butterworth were able to produce granulosa cell and theca cells tumors in mice by irradiation there is only tenuous evidence that irradiation plays a significant role in the genesis of these tumors in the human (Figs 21 4)

During pregnancy and in other states these tumors can undergo luteinization This has given rise *to the term luteoma a term* best avoided for the tumor does not arise from the corpus luteum The phenomenon depends upon the amount of LH present and upon the ability of the cells in question to respond to this stimulus (Figs 25 7) The functional



FIG 21 GRANULOSA CELL TUMOR OVARY This is the pure granulosa cell type with many *Call Exner bodies* no sudanophilic material no theca cells admixed and no feminization $\times 75$



Fig 22 Granulosa cell tumor ovary showing the typical microfollicular pattern (From a drawing in the collection of the late Dr Robert Meyer)

capacity of these tumors is difficult to evaluate Mansell and Hertig[35] have proposed criteria which merit careful attention and further application In recent years it has become apparent that granulosa and theca cell tumors are not entirely innocuous Diddle[8] has reviewed 926 granulosa cell and 263 theca cell tumors in the literature he finds that local recurrence and metastasis can occur in about 20 per cent of the cases after intervals ranging from 10 to 15 years and even longer

In the prepubertal group these tumors when functional may give rise to precocious puberty an estrogen effect However less than 5 per cent of the reported cases arise in this age group Actually about 55 per cent of the granulosa cell and theca cell tumors arise during active menstrual life producing usually the syndrome of menorrhagia and an ovarian mass About 40 per cent arise postmenopausally producing the syndrome of postmenopausal bleeding and an ovarian mass These syndromes are hardly specific merely suggestive many non functional ovarian tumors present clinically in just the same fashion The apparent high incidence of endometrial carcinoma especially noticeable in the postmenopausal group has been much over estimated artifacts of population sampling appear to give falsely high positive data Few of the proponents of the estrogen endometrial cancer hypothesis have corrected their data for the statistically valid concept of multiple primary tumor In most cases coincidental occurrence of tumors does not imply a necessary cause and effect relationship

The Sertoli Leydig cell group of tumors is suggested by Morris and Scully[69] for the arrhenoblastomas or androblastomas They point out that although these tumors are characteristically masculinizing and androgen secreting they may have no clinical effects may secrete no hormones and may rarely elaborate estrogens The evidence for estrogen production by male and androgen production by female gonadal elements invalidates the use of endocrine effect alone as a diagnostic standard However the common clinical denominator is amenorrhea hirsutism clitoral hypertrophy and lowered tessitura When present these effects are largely re

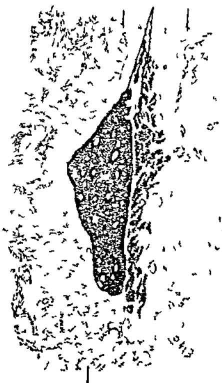


Fig 23 Nest of granulosa cells (Granulosa ballen) in an atretic follicle Can such observations reconcile the conflicting theories of tumorigenesis of Meyer and McKay? (From a drawing in the collection of the late Dr Robert Meyer)

years later McKay Robinson and Hertig[36 7] demonstrated by non specific histochemical methods that the estrogenic activity of granulosa theca tumors could be ascribed to the theca cell The pure varieties of microfollicular granulosa cell tumor are often inert but the pure theca cell tumors are often quite highly estrogenic

Microscopically granulosa cell tumors exhibit a wide variety of patterns—microfollicular trabecular *pseudo tubular plexiform and diffuse (sarcomatoid)* however these tend to overlap and be admixed within any one given specimen Often the apparent stroma of the granulosa cell tumor is in reality composed of small bundles of theca cells surrounding small blood vessels this effect can be demonstrated on occasion by a stain for lipoid in which the theca cells stain positively for intracytoplasmic neutral fat but the prominent epithelial granulosa cell aggregates are negative It must be emphasized that the stains for neutral fat which correlate well with the yellowness of the tumor on section do not stain steroid hormones and correlate poorly with functional manifestations The microscopic appearance of theca cell tumors typically is that of interlacing bundles of short plumpish spindle cells with a variably collagenized stroma On occasion these tumors blend imperceptibly with the inert ovarian fibroma the best criteria for distinguishing a thecoma from a fibroma are the size and shape of cells the amount and distribution of collagen and the presence or absence of stainable neutral fat In general the fibroblasts have longer nuclei with more pointed ends stellate nuclei are found on occasion The cytoplasm of thecomas is more abundant and often shows fine foaminess under reduced illumination Diffuse abundant collagen is more characteristic of fibromas but occasional intercalated hyalinized plaques are found in thecomas Even using these criteria an occasional case will be difficult to evaluate

The histogenesis of these tumors has been debated at length Robert Meyer attributed the origin of *granulosa cell tumors to granulosaballen* nests of granulosa cells which remain in the ovarian cortex after menopause Robinson advanced the idea that they develop from adult graafian follicles Furth and Butterworth thought they arose from disorganized follicles McKay *et al*[38] recently advanced the idea that they arose from atretic follicles McKay also indicated that theca cell tumors might arise in hyperplastic ovarian cortical stroma citing the observation that in many cases the contralateral ovary exhibited such a phenomenon Although Traut and Butterworth were able to produce granulosa cell and theca cells tumors in mice by irradiation there is only tenuous evidence that irradiation plays a significant role in the genesis of these tumors in the human (Figs 21 4)

During pregnancy and in other states these tumors can undergo luteinization This has given rise to the term "luteoma" a term best avoided for the tumor does not arise from the corpus luteum The phenomenon depends upon the amount of LH present and upon the ability of the cells in question to respond to this stimulus (Figs 25 7) The functional



FIG 21 GRANULOSA CELL TUMOR OVARY This is the pure granulosa cell type with many Call Exner bodies no sudanophilic material no theca cells admixed and no feminization × 75



Fig 22 Granulosa cell tumor ovary showing the typical microfollicular pattern (From a drawing in the collection of the late Dr Robert Meyer)

capacity of these tumors is difficult to evaluate Mansell and Hertig [35] have proposed criteria which merit careful attention and further application In recent years it has become apparent that granulosa and theca cell tumors are not entirely innocuous Diddle [8] has reviewed 926 granulosa cell and 263 theca cell tumors in the literature he finds that local recurrence and metastasis can occur in about 20 per cent of the cases after intervals ranging from 10 to 15 years and even longer

In the prepubertal group these tumors when functional may give rise to precocious puberty an estrogen effect However less than 5 per cent of the reported cases arise in this age group Actually about 55 per cent of the granulosa cell and theca cell tumors arise during active menstrual life producing usually the syndrome of menorrhagia and an ovarian mass About 40 per cent arise postmenopausally producing the syndrome of postmenopausal bleeding and an ovarian mass These syndromes are hardly specific merely suggestive many non functional ovarian tumors present clinically in just the same fashion The apparent high incidence of endometrial carcinoma especially noticeable in the postmenopausal group has been much over-estimated artifacts of population sampling appear to give falsely high positive data Few of the proponents of the estrogen endometrial cancer hypothesis have corrected their data for the statistically valid concept of multiple primary tumor In most cases coincidental occurrence of tumors does not imply a necessary cause and effect relationship

The Sertoli Leydig cell group of tumors is suggested by Morris and Scully [63] for the arrhenoblastomas or androblastomas They point out that although these tumors are characteristically masculinizing and androgen secreting they may have no clinical effects may secrete no hormones and may rarely elaborate estrogens The evidence for estrogen production by male and androgen production by female gonadal elements invalidates the use of endocrine effect alone as a diagnostic standard However the common clinical denominator is amenorrhea hirsutism clitoral hypertrophy and lowered tessitura When present these effects are largely re



Fig 23 Nest of granulosa cells (Granulosa ballen) in an atretic follicle Can such observations reconcile the conflicting theories of tumorigenesis of Meyer and McKay? (From a drawing in the collection of the late Dr Robert Meyer)



Fig 24 Granulosa cell tumor ovary luteinized The paler cells resemble luteinized granulosa cells the darker ones resemble luteinized theca cells × 125 (*Inset* × 275)

Note Crop the corner marked x on the low power and set in a nice field from the marked off corner on the high power

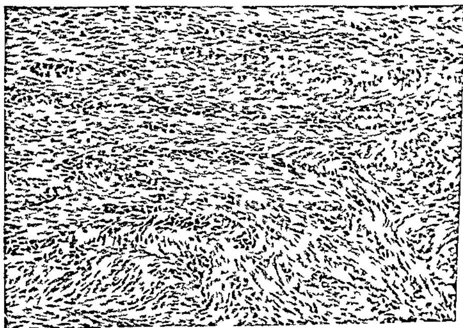


Fig 25 Thecoma ovary The typical fasciculated pattern of plump spindle cells is shown. × 145

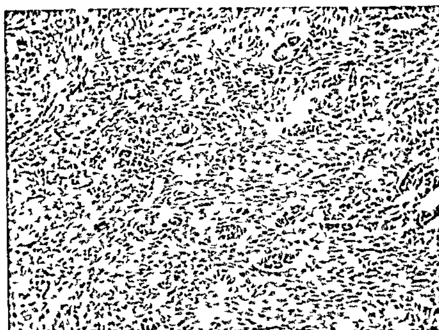


Fig 26 Thecoma ovary This field shows a few tubules in a medium size yellow tumor that was predominantly similar to the one shown in Figure 25 Are these tubules sufficient to classify the tumor as a gynandroblastoma? × 145

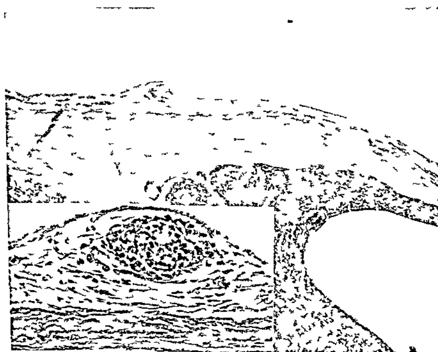


Fig 27 Recurrent granulosa cell tumor 8 years after removal of the primary The inset shows a detail of the subperitoneal nodule

versed after the tumor is removed The common pathologic denominator is a tumor composed of tubules and polyhedral cells with eosinophilic cytoplasm The tubular component resembles that seen in Sertoli cell tumors of the testis In the ovary these tubular formations suggest derivation from the sex cords (Pfluger s tubules) Teilum [62 3] has indicated that some of the tubular varieties of granulosa cell tumor may in fact be feminizing Sertoli tumors and elaborate estrogens The polyhedral cell component is morphologically indistinguishable from the interstitial cell (Leydig cell) of the testis and from the hilar cell of the ovary these cells contain lipoid and often exhibit Reinke crystalloids These latter are specific for Leydig cells irrespective of location Their presence not only eliminates the possibility that the cells in question are paralutein cells of thecal origin but demonstrates the identity of the Leydig cell component of these tumors with the pure ovarian hilar cell tumor

The well differentiated variety of Sertoli Leydig cell tumor is quite distinctive having been first described by Pick in 1905 as an adenoma tubulare testiculare ovarii this is the type classed as Group I by Javert and Finn [7] when the tubules are less well formed the masculinizing effects are often less pronounced and the clinical incidence of malignancy is higher (Group II) in the undifferentiated or sarcomatoid group the morphologic diagnosis may be doubtful and the tumor only assigned to this group by virtue of its clinical effects (Group III) there is an even higher incidence of malignancy in the undifferentiated type One must confess a certain diffidence at classifying tumors composed of a diffuse proliferation of cells which have neither architectural nor specific cellular features to define them Iverson [6] reviewed 91 masculinizing tumors of the ovary including 26 labeled as masculinovoblastoma she established the identity of most of these tumors with the arrhenoid group It is rather droll to observe that several examples of masculinizing tumor have been found in association with pregnancy (Figs 28 30)

The mixed variety of sex cord mesenchyme tumor is the so-called gynandroblastoma a term introduced by Robert Meyer in 1930 He suggested that tumors arising from undifferentiated cells might be hermaphroditic developing partly into male and partly into female elements The term has been used loosely by a number of observers to include a heterogeneous number of tumors including granulosa theca cell tumors with clinical masculinization Sertoli Leydig cell tumors with clinical feminization tumors with mixed or incomplete endocrine manifestations often composed of both granulosa theca cell elements ad



FIG 28 TUBULAR ADENOMA OVARY (This photomicrograph was taken in the 1870 s by Dr Joseph Janvier Woodward at the Army Medical Museum It has been furnished through the kindness of Dr Henry W Edmonds one time Acting Curator)



FIG 29 MASCULINIZING TUMOR OVARY TUBULAR TYPE Pale eosinophilic interstitial cells are seen between the tubular structure × 125

is not elevated in contrast to masculinizing tumors of adrenal origin in which urinary 17 ketosteroids are usually increased The cells are morphologically indistinguishable from the Leydig cells of the testis and from the polyhedral cells of Sertoli Leydig cell tumors of the ovary The precise significance of their close anatomic association with non myelinated nerve fibers is obscure (Figs 31 2)

The term lipoid cell tumor (Fig 33) as used by Morris and Scully[68] is a noncommittal one embracing a number of tumors composed of clear polyhedral cells similar to those in the adrenal cortex varieties of Leydig cells and luteinized cells of granulosal or thecal origin They are usually associated with masculinization when functional The adrenal like varieties are thought to arise from adrenal rests which are not uncommon in the mesovarium or even within the hilus of the ovary (Figs 34 6) The term hypernephroma has been applied to some of these presumably in reference to the fact that the clear cell tumor of the kidney was originally thought to be adrenocortical in origin and that these tumors of the ovary were thought to be of similar origin It is a confusing term implying uncertainty as to renal vs adrenal origin of an ovarian tumor and should be discarded Likewise the term masculinovoblastoma has been applied to some tumors in mixed with Sertoli Leydig cell elements Morris and Scully[69] urge *caution in morphologic evaluation* pointing out that granulosa cells and Sertoli cells are embryologic homologues as are also theca cells and Leydig cells The pseudotubular pattern of some granulosa cell tumors can certainly be confused with Sertoli like tubules one would wish for an unequivocal demonstration of Leydig cells with Reinke crystalloids plus the presence of a typical microfollicular granulosa cell pattern complete with Call Exner bodies before committing one s self firmly to such a diagnosis Certainly a diffuse (sarcomatoid) pattern is not helpful nor are fasciculated spindle cells more than suggestive of theca in such a context

The hilar cell tumor of the ovary is a related but separable entity I would classify it as a variant of the Sertoli Leydig cell group rather than as one of the lipoid cell tumors Sternberg[5] has described such tumors and their clinical effects in detail They occur as spherical well demarcated small masses in the mesovarium or ovarian hilus and are readily distinguishable from adrenal rests in the same location They are composed entirely of polvhedral cells with eosinophilic cytoplasm which contains lipoid lipochrome and crystalloids of Reinke Clinical masculinization is often present but ketosteroid excretion



FIG 30 MASCULINIZING TUMOR OVARY composed predominantly of interstitial cells × 200

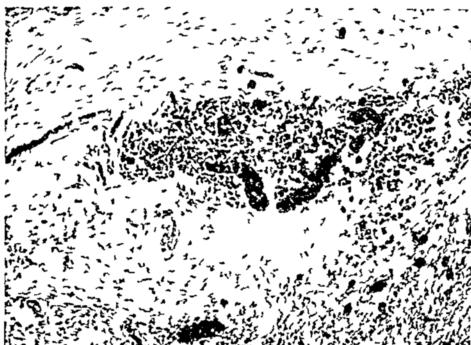


Fig 31 Hilar cell rest ovarian medulla × 115

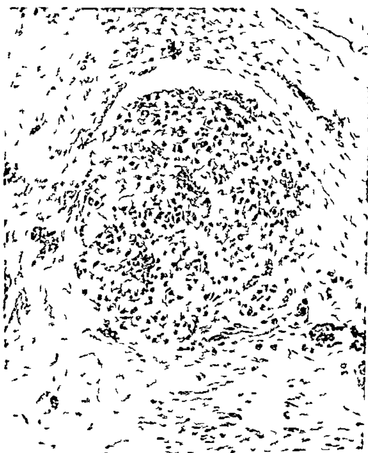


Fig 32 Hilar cell rest ovarian medulla irradiated × 125

this group This would imply a neoplastic prolifera tion of masculine or masculinizing ovoblasts until such time as an ovoblast is identified this term can safely be discarded too In evaluating the relation ship between adrenal hormones and ovarian hor mones and the observations which indicate that some ovarian tumors are derived from adrenal cells the embryologic congruences between the gonad and the adrenal cortex merit attention Both the gonad (male and female) and the adrenal cortex arise as subcoelomic condensations in the neighborhood of the mesonephros one slightly above the other In their earliest stages they exhibit many morphologic features in common which persist even during the phases of differentiation It is not surprising per haps that these two endocrine glands can metabolize acetate and cholesterol into C-21 and C-19 steroids some actually being synthesized in both organs others specific for one or the other The molecular differences between some of these steroids is so slight that it is not difficult to hypothesize the ease of interconversion under suitable physico-chemical con ditions



FIG 33 MASCULINIZING TUMOR OVARY composed of lipid filled polyhedral cells resembling vaguely those of the adrenal cortex

The common tumor of the germinoma teratoma group is the disgerminoma of the ovary which is analogous to the seminoma of the testis This tumor rarely produces any clinical picture recognizable as an endocrinopathy though bilateral destruction of the ovarian parenchyma may lead to defeminization However in a few instances their presence has been accompanied by elevated FSH values and by positive pregnancy tests (i e elevated gonadotropin levels) which have fallen to normal or absent after the tumor was removed The origin of the FSH is obscure the presence of HCG would imply the presence of trophoblast somewhere in the tumor but this has rarely been confirmed histologically In certain cases

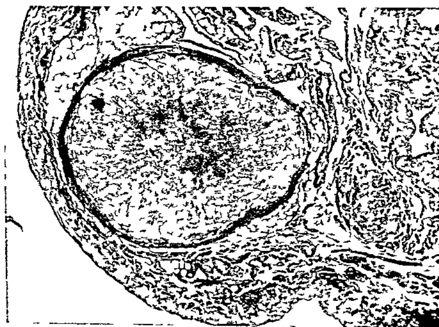


FIG. 34 ADRENAL REST MESOVARIUM There is a well-defined capsule and there is a cortico-medullary arrange ment of the cells It is a true rest × 40



Fig 35 Detail of figure 32 showing a rudimentary zona glomerulosa and zona fasciculata × 125



Fig 36 Mesovarial rest type undetermined The capsule is not well formed and there is no cortico medullary arrangement The cells are in the form of small clusters possibly syncytial The presence of clear cytoplasm does not indicate adrenal origin × 145 (*Inset* × 355)

of teratocarcinoma of the ovary both syncytiotrophoblast and cytotrophoblast has been identified (Fig 37) the presence of a positive HCG test is not surprising In some benign ovarian teratomas there has been a predominance of thyroid tissue even to the point of obliterating other teratoid elements This is the well known struma ovarii while it is usually functionally inert an occasional case gives evidence of function In one such case hyperthyroid symptoms recurred two years following subtotal thyroid

ectomy for thyrotoxicosis Radioactive iodine tracer studies indicated accumulation of the substance in a pelvic mass which proved to be struma ovarii with microscopic evidence of hyperplasia colloid vacuolization and depletion etc The symptoms of recurrent thyrotoxicosis were relieved by ovariectomy Morris and Scully[68] illustrate a case in which the thyroid epithelium was transformed into malignant tissue

Scully has reviewed the problem of the gonadoblastoma a rare tumor composed of proliferated germ cells plus admixture of sex cord and mesenchymal derivatives The term gonadoblastoma indicates the presence of most of the essential cellular



FIG 37 TERATOCARCINOMA OVARY WITH TROPHOBLASTIC DIFFERENTIATION Most of the trophoblast resembles syncytiotrophoblast × 100

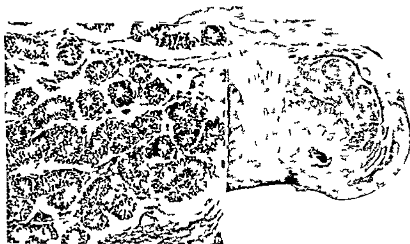


FIG 38 OVOTESTIS The ovarian portion is seen at the left and contains developing follicles surrounded by theca and ovarian stroma as well as numerous ova The testicular portion is at the right and shows testicular tubules with a few nests of interstitial cells × 8 (*Inset* × 100)

elements of the gonad As in the problem of gynandroblastoma the problem of classifying the cellular elements accurately is most difficult Scully indicates the possibility that this sort of tumor might represent a form of intersexuality If so there is a possibility that it may be related in some way to the rare occurrence of an ovotestis (Fig 38)

The tumors with functioning stroma include the Brenner tumor the Krukenberg tumor (metastatic adenocarcinoma) and the cystadenofibroma Rarely in such cases the ovarian stroma assumes a thecal appearance the cells being rather plumper than average with more cytoplasm Some patients have shown evidence of virilism others have shown evidence of estrogen production These examples merely illustrate the fact that under certain conditions the ovarian stroma can be induced to elaborate steroid hormones

It is evident from this brief discussion that there are many complexities regarding endocrinologically active ovarian tumors which have not been solved Detailed consideration is beyond the scope of this chapter A full treatment of present day knowledge in this field can be found in the monograph by Morris and Scully[68] which is well illustrated and carefully documented

3 The Pale White Ovary

The deliberately noncommittal term pale white ovary has been selected to describe the ovary often associated with the Stein Leventhal syndrome but also found in such overlapping conditions as metropathia hemorrhagica and some examples of pseudo virilism The ovarian changes are to some extent a common denominator to these clinical states but the syndromes can occur in the presence of relatively normal ovaries as well The ovarian lesions have been described diversely as polycystic ovaries thecomatosis hyperthecosis thecal hyperplasia with diffuse luteinization and other terms as well This confusing verbiage is descriptive rather than explanatory indeed it appears that no single explanation can account for the wide range of clinical manifestations the variable changes in the ovary and the inconstant endocrine observations

In the full blown picture the ovaries are moderately and diffusely enlarged preserving the contour of the normal gonad The external surface presents with a peculiar oyster white color representing a cortex with a variable degree of fibrous thickening Through the thickened cortex a number of translucent cystic structures 2 to 15 mm in size present as small bosses often faintly bluish in color these represent cystically expanded graafian follicles which have not ruptured are still filled with liquor folliculi but are undergoing atresia Often these atretic follicles are surrounded by thick mantles of luteinized theca interna Somewhat deeper in the cortex are collapsed atretic follicles with a core of delicate fibrous tissue these also are often surrounded by a mantle of luteinized theca interna Intervening between the atretic follicles is a diffuse hyperplasia of the theca externa and cortical stroma the hyperplastic thecal and stromal cells usually are plumper than average and may have larger more vesicular nuclei than in the non hyperplastic state Scattered in the zone of thecal hyperplasia are irregularly distributed nests of large polyhedral cells with clear cytoplasm presumably representing luteinization of the stroma Corpora albicantia are infrequent in any case and absent in many

One of the keys to this lesion is the absense of the usual sequence of follicular rupture ovulation and the formation of a corpus luteum To what extent this is a consequence of the barrier of a thickened collagenized tunica albuginea is difficult to state Possibly such a barrier serves to delay follicular rupture until the ovum is past its prime In such cases however true ovulation occasionally does occur The diffuse luteinization implies an excess of LH but this has not been satisfactorily demonstrated in most cases The hyperplasia of theca and cortical stroma imply excessive elaboration of estrogens but under certain circumstances increased androgen production is also possible To what extent persistently elevated LH levels inhibit ovulation cannot be assessed

The clinical syndromes associated with such ovaries are likewise ambiguous Over the past two decades Stein and Leventhal[58] have described a syndrome in young women characterized by amenorrhea or severe oligomenorrhea hirsutism and obesity The syndrome may be present from the time of menarche presenting as a fertility problem or it may develop following one or more successful pregnancies However the symptoms are certainly not specific many women have scant menses are fat hairy and otherwise unprepossessing for non endocrinologic reasons By way of contrast still another group of women present with severe often uncontrollable menometrorrhagia at any age from 15 to 35 This group has been labeled metropathia hemorrhagica a misleading term for the hemorrhage is usually not from a diseased womb Still other women present with signs of virilism Similar lesions have been found in the ovary in all three groups however many women who exhibit similar clinical pictures have normal ovaries

Examination of the endometrium in such cases can be equally ambiguous Most of the cases in which amenorrhea of severe oligomenorrhea is a prominent feature exhibit an atrophic endometrium a significant percentage however exhibit some de-

gree of hyperplasia either diffuse of focal not uncommonly with some degree of cellular and architectural atypism Most of the cases with menometrorrhagia exhibit some degree of hyperplasia likewise variable in extent and degree but a significant percentage apparently bleed profusely from an atrophic endometrium The cases with pronounced pseudo-virilism usually have an atrophic endometrium often a hypoplastic uterus to match but exceptions have been reported A significant number of cases have undergone repeated curettage over a period of years exhibiting progressively more severe degrees of glandular atypism at hysterectomy some have been found to harbor well differentiated endometrial adenocarcinomas often with a mixture of squamous change (adenoacanthoma) In some cases endometrial cancer has been found in association with the pale white ovary shortly after the patient first came to medical attention Almost invariably the women in this group who develop endometrial cancer do so before the age of 40 This is a small group but a significant one it represents the most suitable population for demonstrating the role of endocrine imbalance in the genesis of endometrial cancer however one must be hesitant in imputing such carcinogenesis to estrogens *alone* There is considerable evidence that such ovarian lesions are not uniformly estrogenic some may even be androgenic overemphasis on estrogens serves only to divert attention from the more generalized endocrine disturbance and deprecates the possible role of other steroids in the carcinogenetic sequence

In many instances comparable clinical pictures can be found in association with adrenocortical hyperplasia the ovarian lesion may be present in such individuals but more commonly it is not There is some evidence that stromal luteinization is more extensive in cases with adrenal hyperplasia than in those in which the pituitary has been hinted at as the seat of the derangement However the correlation is not well established for this point as indeed it has not been established for any of the significant features of the syndromes or the lesions

The laboratory evaluation of the patient s endocrine status has probably been less helpful as a diagnostic tool in these circumstances than in any other condition Extensive studies have failed to show any characteristic pattern in urinary hormone excretion The various tests of thyroid function have usually been within normal limits FSH assays likewise are generally normal In a few cases urinary pregnanediol has been found to be elevated whether this is due to the use of methods in which chromatographic separation was not carried out and the pregnanediol is largely pregnanetriol remains to be elucidated Certainly increased pregnanetriol excretion would imply adrenocortical overactivity 17 ketosteroid values however are usually normal In a few cases 11 oxysteroids have been elevated but this likewise is inconstant

Although diagnosis is not completely satisfactory a method of therapy has been devised which has been gratifying in many cases albeit its mechanism remains obscure This consists of the so called wedge resection of the ovaries in which a sizable wedge of thickened cortex is removed and the edge of the incision into the gonad sutured together It is common practice to puncture the cysts which are visible at the capsular surface at this time Approximately 70 per cent of the patients so treated have been able to re establish a reasonably normal menstrual cycle In some series as many as 40 per cent of such patients have become pregnant No satisfactory explanation has been found for the success of surgical therapy and the failure of endocrine therapy in this syndrome

Many of the unanswered questions in this syndrome are implicit in a well documented example of 'hyperthecosis with masculinization [6] not only are the possible pitfalls of clinical diagnosis presented but the difficulties of pathologic interpretation are likewise set forth Certainly the mechanism of androgen elaboration by theca cells luteinized or not needs clarification before our understanding increases

It is apparent that in these patients we are dealing with a set of interrelated phenomena involving the pituitary two of its target organs the adrenal cortex and the ovary plus the target organ of the ovary the endometrium as well as the adrenocortical gonadal interrelationship The lesions in the end organs probably reflect the lack of co-ordination and loss of synchrony in the production of hypophyseal hormones depends in part on reciprocal inhibition by the hormones produced in the ovary and the adrenal cortex Persistent excess or persistent deficiency of any one important gonadotropin or steroid may lead to a self perpetuating loss of cyclicity Lopez s law regarding the navigation of small sailing craft might well apply to the endocrine stimulus end organ response You have to know not only where the wind is blowing but how your sails are set

4 Adreno-genital Syndromes

If the details of correlative endocrine pathology are deficient in regard to previous topics discussed the lack of information is maximal in respect to the congeries of clinical phenomena lumped together as adreno genital syndromes The sporadic nature of such cases and the limited methods for their study are the principal factors in our lack of understanding It is only in recent years that any effort has been made to consider them as something beyond a *lusus*

naturae and to explain their occurrence and some of their features on a rational basis Jolly s[2] study of *sexual precocity emphasizes that precocity may be* either constitutional or pathologic Constitutional precocity is merely the development of physiologic and anatomic puberty prior to the lower limit of chronologic age accepted as normal therefore it is merely an expected statistical deviation in respect to time beyond the usual limits of standard deviation and is the exact counterpart of delayed puberty It is not an endocrine disorder and should not be treated as such Truly pathologic precocity is found in association with certain intracranial tumors notably those involving the hypothalamus adrenal tumors and hyperplasias and gonadal tumors notably feminizing mesenchymal tumors such as the granulosa cell tumor of the ovary To this must be appended a number of miscellaneous causes

Sexual precocity is more frequent in girls than in *boys and the commonest cause is constitutional* precocity Unless pseudohermaphroditism is present adrenal tumors and hyperplasias are uncommonly found Granulosa cell tumors are very rare In constitutional precocity there is true precocity early puberty is real puberty and both ovulation and regular menses occur even pregnancy has been known to take place In pathologic precocity there is rarely true ovulation the precocity is due to stimulation of secondary sex characteristics such as mammary hypertrophy development of pubic and axillary hair the development of a simulated adult habitus etc If the primary lesion is an intracranial neoplasm it may be a pinealoma but other types of tumor have been found the encroachment of the tumor on the hypothalamus and disturbance of the hypophyseal hypothalamic pathways appear to be the important factors in determining whether or not a given tumor will produce precocity If the lesion is located in the adrenals it may be a tumor notably adrenal cortical carcinoma cortical adenoma hyperplasia or merely hyperfunction sans lesion In adrenal precocity 17 ketosteroid excretion is elevated However adrenal lesions are more common in boys than *girls* If the lesion is ovarian an estrogen producing granulosa cell tumor is possible

Jones and Jones[28] have studied the gynecologic aspects of adrenal hyperplasia They define four groups of patients (1) female pseudohermaphroditism due to congenital adrenal hyperplasia (2) postnatal virilization due to adrenal hyperplasia (3) *postpubertal* hirsutism oligomenorrhea with and (4) without elevated 17 ketosteroid excretion—possibly due to adrenal hyperplasia or an allied disorder The latter two classes are variants on the Stein Leventhal syndrome viewed *from the standpoint of* adrenal disturbance In female pseudohermaphroditism there is hyperplasia of the zona reticularis of the adrenal cortex this is also found in Cushings syndrome and seems to be associated with masculinizing effects notably hirsutism Similar changes of lesser degree were found in the other groups as well Associated with the hyperplasia of the reticularis is lipid depletion of the fasciculata and *glomerulosa* sites of adrenocorticoid formation To what extent such observations are significant awaits evaluation Certainly the ovaries mirror the changes in the adrenal poorly In some instances the ovarian cortex is devoid of follicular elements while in others there is evidence of follicle development to the stage of *antrum formation but without rupture and hyper*trophy of the cortex and theca As in the Stein Leventhal syndrome the relation of hormones to specific changes in end organs is an enigma wrapped in a mystery

The endocrine control of sexual differentiation in embryo is well known Burns has produced an infinite variety of modifications on the form of the urogenital apparatus in the opossum by treating the immature marsupial with estrogens androgens in series in combination by withdrawal and in association with other compounds It is reasonable to infer that both adrenal and ovarian hormones act as important organizers in the embryonic development of the sexual apparatus of the human as well However the rarity of these cases makes them *sui generis* Likewise the occurrence of an ovotestis has been observed in the human Though histologically fascinating its significance remains obscure

VI SOME ENDOCRINE ASPECTS OF PREGNANCY

If we follow accepted normative behavior ovulation occurs between the 13th and 15th days of the menstrual cycle and fertilization can occur in the oviduct any time from the 15th through the 18th day and implantation occurs after a more variable period usually between the 22nd and 26th day *The fertilized* ovum lies free in the tubal lumen and endometrial cavity for several days during which time it undergoes division and multiplication until it becomes a blastocyst characterized by a central cavity with a cellular condensation at one pole known as the embryonic pole Implantation occurs in the blastocyst stage and *normally the embryonic pole is* the point of contact and implantation A necessary prerequisite for implantation is a suitably prepared endometrium i e one in which the peak of secretory activity has passed but in which stromal edema persists and predecidual transformation of stroma is beginning The cells at the embryonic pole form the *primitive trophoblast* even at five days after implantation the syncytiotrophoblast is on the outer aspect of the ovum and cytotrophoblast is inside The ovum

is in the previllous stage for about 14 days after implantation i e about one week after the date of the first missed period After that chorionic villi develop and fetal angiogenesis proceeds These are the rough outlines but the limits of deviation are narrow If there is significant failure of any of the elements abortion will ensue Abortion may be due to blighted ova faulty implantation or inadequate endometrial control It is implicit in the management of human sterility that efforts must be directed at maintaining a normal endometrium (Figs 39 40)

Substantially all the endocrine effects in pregnancy can be traced to the development of the placenta a gland of internal secretion which is of fetal origin and limited life span The placenta elaborates estrogens progesterone and chorionic gonadotropins All the naturally occurring human estrogens (estradiol estriol and estrone) have been isolated from the placenta and recently progesterone has been isolated as well Chorionic gonadotropin is a glycoprotein with a molecular weight of about 80 000 It is not the equivalent of pituitary gonadotropin albeit they share certain biologic properties in common Both gonadotropins stimulate production of estrogens and progesterone however chorionic gonadotropin does not stimulate ovulation or corpus luteum formation as does pituitary gonadotropin The steroid hormones of the placenta are formed in the syncytiotrophoblast the gonadotropins in the cytotrophoblast or Langhans layer In the normal human placenta the Langhans layer regresses during the second trimester of pregnancy and chorionic gonadotropin levels in serum and urine fall concomitantly The maintenance of a normally implanted ovum during the first six or eight weeks depends primarily upon the adequacy of the decidua through which it is nourished Decidua of pregnancy is stimulated and maintained at first by the progesterone from the corpus luteum of pregnancy and then by the progesterone from the syncytiotrophoblast In contrast to chorionic gonadotropins estrogen and progesterone levels increase in the serum and urine progressively throughout gestation until just prior to the onset of labor Some observers feel that steroid hormone withdrawal as the syncytiotrophoblast rapidly involutes is an important factor in determining the onset of labor Courrier has stated the situation with clarity Estrogen is the hormone of the woman it assures the development of the genital and mammary apparatus progesterone is the hormone of the mother it is indispensable for reproduction

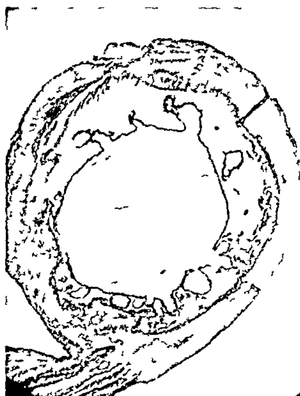


FIG 39 ABNORMAL PREGNANCY BLIGHTED OVUM The embryonic disc is absent The implantation was superficial and pseudo-polypoid Chorionic villi are hypoplastic as is the trophoblast The patient aborted 3 weeks after this missed period × 30

1 *Pregnancy Tests*

In essence the usual laboratory test for pregnancy is a limited bioassay for the presence of chorionic gonadotropin While true positive results have been obtained as early as one day after the missed period the peak of accurate positive results is not reached until two weeks later i e three weeks after implantation A positive result is a function chiefly of the quantity of chorionic gonadotropin elaborated by the cytotrophoblast and as is evident an accurate diagnosis can be made while the ovum is in the previllous stage The desiderata of such a test include absence of false positive tests and a high percentage of true positive ones Any test which is less than 95 per cent accurate in true positives from two weeks after the first missed period to about the middle of gestation is valueless During the latter half of pregnancy false negative results may be obtained if the chorionic gonadotropin level is sufficiently low This can become of clinical importance if pregnancy is suspected at or about the expected time of the menopause particularly in obese patients in whom physical signs may be difficult to elicit Although the HCG titer is low in the last half of pregnancy after the Langhans layer has disappeared from the chorionic villi enough HCG is elaborated by cytotrophoblast in the basal plate to give positive results in a test of suitable sensitivity

The usual biologic tests for pregnancy are four



FIG 40 PLACENTAL SITE AT 2½ MONTHS GESTATION Multinucleated cells are present both trophoblastic and decidual in origin There is decidual transformation of the adventitia of an endometrial venule × 200

in number and like the *contrade* of Siena are named for specific animals The mouse test (the true Aschheim Zondek test) involves the use of five mice each of which is given six successive injections of urine over a two-day period the animals are sacrificed four days after the start of the test The presence of one hemorrhagic follicle or one corpus luteum in the ovary of one of the five immature virgin female mice is interpreted as a positive reading This has never been popular in America because of the number of animals and the number of injections The rabbit test (Friedman test) involves the use of a mature virgin female rabbit and the end point is the development of a ruptured hemorrhagic follicle in the rabbit s ovary the day following injection of the suspected urine If the test is negative the rabbit may be re-explored the following day Although the rabbit test is quicker than the mouse test and involves only one injection into one animal the end point is not sharp Great care must be taken to discriminate between ruptured and unruptured follicles (a frequent source of error) and the difficulties

in maintaining the virginity of a colony of rabbits are legion Largely because of these difficulties two tests utilizing amphibia have been developed The toad test developed by Hogben utilizes *Xenopus laevis* the South African clawed toad and the end point is the extrusion of eggs 8 16 hours after the injection of urine which has been concentrated by acetone precipitation This test has the advantage of an all or none end point rarely giving a false positive result Its accuracy is from 96 to 100 per cent Although the toads are easier to maintain than rabbits or mice they are somewhat difficult to procure and rather expensive This last factor is offset by the fact that each toad can be used about 10 times a year The frog test has been developed in America and utilizes a male frog *Rana pipiens* the end point being spermatogenesis two to four hours after injection of urine from a pregnant female Like the toad test it has an all or none end point and false positive results are extremely rare False negative results are not uncommon with low chorionic gonadotropin titers particularly during the first two weeks after the first missed period and after the middle of the second trimester However the rapidity of the result the ease of the test and the inexpensive procurement and maintenance of the frogs have made this test most popular during the past decade

Another test for chorionic gonadotropin is its separation by paper electrophoresis from other protein constituents of the serum Its application has not yet been widespread enough to permit evaluation

2 Hydatidiform Mole and Chorionic Tumors

A hydatidiform mole can be defined as a retained blighted ovum in which the primary malformation was failure of continued placental angiogenesis The failure of fetal circulation and the persistence of maternal circulation favor the accumulation of fluid and electrolytes within chorionic villi producing the typical grape like vesicles The majority of hydatidiform moles are aborted or evacuated without sequelae In a minority of cases there is a tendency for proliferative response by the trophoblast The proliferation may be benign or malignant Hertig [9, 21, 8] has shown that it is possible to classify proliferated molar trophoblast and predict with a limited degree of accuracy which moles are more likely to be followed by clinically significant sequelae These sequelae include syncytial endometritis (postmolar endometritis) chorioadenoma destruens and choriocarcinoma

Syncytial endometritis is merely an exaggerated reaction at the placental implantation site following evacuation of the mole It is analogous to postabortal endometritis and postpartum endometritis and is almost a physiologic response to the presence of retained placental site giant cells and decidua Its symptom is recurrent or persistent bleeding after the passage or evacuation of a hydatidiform mole and its treatment is curettage Microscopic examination of the curettings usually establishes the diagnosis but false diagnoses of choriocarcinoma have been made because of the bizarre appearance of the retained trophoblastic cells the presence of multinucleated decidual cells and visible vascular invasion by trophoblast It cannot be too strongly emphasized that trophoblast is a fetal tissue foreign to the maternal organism and one of its principal normal properties is its ability to invade blood vessels The dilatation of blood vessels at the placental site the transformation of their adventitia to decidual cells and alterations of the ground substance of the vessel wall are easily recognized in any examination of a normal placental site These are accentuated when there is an accentuated tendency for trophoblastic proliferation consequent upon a malformation It has not yet been determined whether the ability of trophoblast to invade blood vessels is due entirely to its hormones or whether an enzymatic action upon the ground substance of placental site vessels is implicated as well

Another effect of chorionic tissue can be seen in endometrial glands Arias Stella [1] has described and illustrated a variety of atypical features of cell morphology including marked secretory activity cellular reduplication nuclear hypertrophy and hyperchromasia These changes are common to abortions tubal pregnancies hydatidiform moles and chorionic tumors Presumably they represent a response to hormones liberated by trophoblast However atypical the cellular changes may appear they are not to be confused with the changes of endometrial malignancy

Chorioadenoma destruens is a benign but locally invasive tumor of the uterine wall It presents in the form of a mural or intramural nodule composed of hydatidiform chorionic villi surrounded by masses of proliferated trophoblast Vascular invasion by trophoblast is commonly seen at the periphery of the tumor Unlike choriocarcinoma which can develop after a normal pregnancy chorioadenoma destruens can only develop after a hydatidiform mole It is sometimes difficult to evaluate the neoplastic quality of the surrounding trophoblast this embryonic tissue always looks more malignant than it really is If there is recurrent or persistent bleeding following the passage or evacuation of a mole and curettage yields a combination of hydatidiform villi and proliferated trophoblast one can be reasonably certain that one is dealing with either a retained mole or a chorioadenoma destruens If the former is present careful curettage will be effective If a chorioadenoma destruens is present subsequent hysterectomy will



FIG 41 HYDATIDIFORM MOLE A molar villus is seen invading a decidual sinusoid There is no proliferation of trophoblast ×75



FIG 42 CHORIOADENOMA DESTRUENS The proliferated trophoblast around a molar villus is invading a myometrial vessel The lesion is benign ×50

usually be necessary to extirpate the villi and trophoblast which have burrowed into the myometrium If not removed a chorioadenoma destruens can perforate the uterine wall and produce massive intraperitoneal bleeding Rarely if ever does a choriocarcinoma develop from a chorioadenoma destruens but such transition forms have been reported The reports are not well documented or convincing but the theoretical possibility remains (Figs 41-43)

Choriocarcinoma (chorion epithelioma) is the true malignant tumor of trophoblast[46] It develops most commonly after a hydatidiform mole (a true example of a *Geschwulst* following a *Missbildung*) but it can arise after a spontaneous abortion a tubal pregnancy or an apparently normal intra uterine pregnancy Chorionic villi are absent in a choriocarcinoma and the microscopic diagnosis rests entirely upon the recognition of neoplastic trophoblast It is unwise to attempt the diagnosis upon curettings obtained at the time a hydatidiform mole is evacuated In general it is a rapidly fatal malignancy producing widespread metastases to the lung brain and liver However prompt recognition and early hysterectomy have resulted in long term cures Caution must be exercised in evaluating reported cures for recurrence and death have followed as much as seven years later the tumor being silent during the interval An occasional case with pulmonary metastasis may respond to hysterectomy and resection of the pulmonary lesion Choriocarcinoma may be present in malignant

ovarian teratomas as one of the varieties of tissues present This is more common in the male gonad where it may even predominate clinically and histologically

All these lesions of trophoblast share in common the ability to elaborate chorionic gonadotropin Consequently any sensitive biologic test for this hormone will be positive In general such tests are positive for four to six weeks after a normal spontaneous abortion in the first trimester It is not surprising therefore that they may continue to be read as positive for eight to ten weeks after the complete evacuation of an innocuous hydatidiform mole This emphasizes the importance of performing quantitative gonadotropin tests as a base line as soon as a trophoblastic lesion is suspected A fall in titer at four weeks is suggestive of a continued innocuous course but persistence or increase of the titer suggests that one of the clinically significant sequelae may be developing However the value of the gonadotropin test is limited and it is no substitute for careful observation of the patient s clinical course and repeated examinations Likewise careful evaluation must be made of pathologic material in the light of the clinical history and with due regard for the limitations upon the accuracy of prediction of the possible behavior of this most unpredictable tissue

It is not uncommon to find that the ovaries of a patient with syncytial endometritis chorioadenoma destruens or choriocarcinoma are enlarged This enlargement is usually bilateral and symmetrical It is due to the formation of theca lutein cysts in large number as a response to the elevated circulating gonadotropin Neoplastic trophoblast rarely if ever metastasizes to the ovaries unless there is obvious anatomic evidence of a hemorrhagic metastatic nodule in one of the enlarged ovaries they should not be removed when laparotomy is done for evacuation of a mole by hysterotomy when hysterectomy is done for chorioadenoma destruens or even for choriocarcinoma

Among the phenomena associated with trophoblast is that of benign transport It is not uncommon to find isolated cells of syncytiotrophoblast in the capillaries of the lungs of women who have died during the last trimester of pregnancy It is also possible that such benign transport occurs early in pregnancy and indeed throughout pregnancy The recent publication of an example of a fragment of decidua in the lung [47] and another report of pulmonary endometriosis [8] with decidual transformation during pregnancy impels one to speculate regarding the possible results of benign transport of trophoblast should a few stray syncytiotrophoblastic cells be implanted in the lung This might serve as a possible explanation for the rare case of choriocarcinoma apparently primary in the lung some time following a normal pregnancy

3 The Ovary in Pregnancy

Changes in the corpus luteum during pregnancy have been discussed above A thorough review of



FIG 43 CHORIOCARCINOMA The pattern of neoplastic cytotrophoblast cell capped by neoplastic syncytiotrophoblast cells all growing in a plexiform pattern is most characteristic X 75

this subject is furnished by Nelson and Greene[42] who point out that specific criteria for distinguishing among a corpus luteum of pregnancy a corpus luteum of menstruation and a corpus luteum cyst are not clearly defined They also indicate that our present day knowledge concerning the nature of the K cell and the role of theca lutein cells is far from satisfactory Their description of the occurrence and origin of the ectopic decidual *response from the* subcoelomic mesenchyme leaves nothing to be desired

4 Eclampsia

The role of hormones in eclampsia has long been an open question as indeed are most of the aspects of this mysterious disease Eclampsia like syndromes have been produced in animals with a plausible similarity to both the clinical aspects of the syndrome and to some of the lesions seen in the human female One such technique involved the administration of desoxycorticosterone acetate to pregnant animals first rendered hypertensive by unilateral renal arterial compression *Another method* involved the administration of large doses of progesterone to animals during the last trimester Unfortunately neither desoxycorticosterone nor progesterone seem significantly implicated in the etiologic background of human eclampsia Smith and Smith[53 55] have extracted a toxin from human menstrual blood which produced a toxemia like syndrome in experimental animals the syndrome could be prevented by the concomitant administration of a protective pseudo globulin Increased yields of the toxin could be obtained if progesterone was administered prior to menstruation Unfortunately the pathologic changes produced in these animals have never been documented Possibly of greater significance is their observation that eclamptic patients exhibit with considerable uniformity increased gonadotropin levels in serum and urine often accompanied by a subnormal estrogen level in urine and serum[54] They argue that withdrawal of hormonal support from the pregnant as from the nonpregnant uterus may result in the formation of a menstrual like toxin in the placenta thereby precipitating the toxemic or eclamptic episode[56] *However in evaluating such* theories we must remember that there is a marked increase in the excretion of both estrogens and progesterone during the two weeks before term and that absolute quantities of these steroids need not be so significant as their proportion to each other not only at the time of a given determination but throughout the course of the gestation in question Fluctuations in rate of pregnanediol excretion or in serum *estrogen* levels viewed in the perspective of several previous determinations on the same patient may be of clinical significance in anticipating toxemic crises

Degenerative changes have been described in the syncytiotrophoblast of the placenta in eclampsia these are in the nature of premature involution of the gland and may be related to the lowered estrogen levels described by Smith and Smith It is not clear however that they represent a primary defect *possibly the premature degeneration of the tropho*blast in the last trimester is secondary to degenerative lesions in decidual blood vessels such as accumulations of lipophages and fibrinoid alteration of the vessel walls However as is the case with most of the lesions encountered in human eclampsia[11] they are inconstant in their occurrence and ambiguous in interpretation

5 *Diabetes Mellitus*

Diabetes mellitus is a systemic pluriglandular disease When a known diabetic becomes pregnant endocrine equilibria are often stretched to the breaking *point hence the increased maternal* and fetal risk White and her associates[41] found empirically that pregnant diabetic women had consistently lowered serum estradiol levels and urinary pregnanediol levels Their plan for management includes careful control of the diabetes and regular parenteral administration of progesterone in large doses and oral stilbestrol in large doses as well as delivery by elective caesarean section at about 36 weeks of gestation While the careful medical supervision afforded these patients is partly responsible for their low maternal mortality and morbidity their favorable fetal salvage can in part be attributed to the role of the steroid hormones in preventing the premature degeneration of the placenta so commonly found in diabetic pregnancies not given these hormones Also significant is the early date of delivery However the criteria for prematurity in an infant born to a diabetic mother are different from those applicable to one born to a nondiabetic mother and *respiratory disease of the* newborn is a serious problem in this group Also the steroid hormones appear to have some beneficial effect upon diabetic retinitis which usually is aggravated by pregnancy

6 The Endocrine Effects of Pregnancy upon the Maternal Organism

The effects of pregnancy upon the reproductive organs have been dealt with under appropriate headings above The other endocrine phenomena merit some attention albeit many aspects of their nature are not clearly understood For example it is well known that the thyroid gland enlarges to an appreciable extent in the majority of pregnant women

that there is an increase in basal metabolic rate and that there is a mild but true hyperplasia of the gland which is reversible when gestation terminates However it is not established that this is a consequence of increased TSH elaboration by the anterior pituitary although there is no reason to discredit this explanation Likewise there is a detectable increase both in parathyroid size and function teleologically this is significant in mineralization of the fetal skeleton and it certainly is significantly related to negative maternal calcium balance but the details of this relationship need elucidation Likewise there is thickening of the adrenal cortex during pregnancy particularly in the zona fasciculata however which of the corticoids are elaborated in increased amounts and the significance of this observation are not clearly definable In the anterior pituitary there are many changes during pregnancy the basophiles progressively develop into large granular cells until the middle of pregnancy but become progressively depleted of granules during the latter half the so-called pregnancy cells are highly acidophilic but revert to their original chromophobe state after pregnancy However the significance of these easily recognized changes is debatable

Lastly in pregnancy pre existing vascular lesions tend to become more severe occasionally presenting as acute catastrophic clinical emergencies Such examples as toxemia secondary to pre-existing hypertension and dissecting aneurysm of the aorta can be considered Although the latter lesion is usually found in men over the age of 40 when it occurs in women under the age of 40 over 50 per cent of the cases are associated with pregnancy usually at or near term or shortly thereafter Whether or not there is a generalized effect of the endocrine changes in pregnancy upon the walls of blood vessels is an open question Covan finds evidence to support the theory that most of the vascular changes in eclampsia are endocrine in origin

7 The Endocrine Effects of Pregnancy upon the Fetal Organism

The fetus lives in the total metabolic *milieu* of the maternal circulation insofar as its elements traverse the placental membrane There is little room for doubt that the smaller molecules such as thyroxin adrenalin and the steroid hormones of the adrenal cortex and gonads can traverse the placenta and stimulate the same end organs in the fetus as in the mother The histologic effects are not always so readily demonstrable as in the case of secretory activity and decidual reaction in the neonatal endometrium but the metabolites of such hormones are recoverable from the urine of newborn infants in appreciably increased amounts at birth and progressively lesser amounts during the first week or so of life The excretion of maternal hormones and reversion to autonomous endocrine equilibria are referred to in the French literature as *la crise hormonale du nouveau ne* but the significance remains putative It has yet to be demonstrated how the presence of maternal hormones within the fetus prepares it for the passage through the birth canal or to cope with neonatal life As a biologic phenomenon the endocrine readjustments after birth are relatively slow when compared with the respiratory and circulatory readjustments which must be made almost at once or not at all Conversely they are relatively fast when compared with certain hematologic and immunologic processes such as the loss of fetal antibodies and the development of its own antibodies by the newborn

One of the important endocrine readjustments which occurs after birth is the involution of the fetal adrenal cortex and the development of the so-called adult adrenal cortex which is present as a thin rim at birth Precisely what hormones are elaborated in the fetal cortex remains undetermined However Knobil[30] has shown that ACTH can traverse the placental membrane Whether this is true of other hypophyseal tropic hormones remains undetermined However the observation of follicle maturation in the ovary to the point of antrum formation and the luteinization of theca interna as well as the hyperplasia of testicular interstitial cells can possibly be ascribed to gonadotropic action whether hypophyseal or chorionic is not determined

The essential difficulty in evaluating many of these observations lies in the fact that we have to deal with two distinct endocrine systems maternal and fetal which are separated from each other by an interposed gland of internal secretion the placenta which at term is involuting but still partially active For example in attempting to locate the source of the progesterone responsible for the decidual reaction in the neonatal endometrium the possibilities include the maternal corpus luteum of pregnancy the maternal adrenal cortex the placental syncytiotrophoblast and the adrenal cortex of the newborn either adult or fetal It is possible that the use of radioactive hormone tracers may elucidate these problems In general pregnancy can be viewed as a naturally occurring experimental situation in which not only are the homeostatic equilibria adapted to new norms but also new equilibria are interposed The occurrence of pathologic events in pregnancy can modify these equilibria and the modifications can be interpreted not only by observations of disordered function but by visible alterations of tissue and cellular structure

BIBLIOGRAPHY

1 Arias Stella J 1954 Atypical endometrial changes associated with the presence of chorionic tissue Arch Path 58 112 28
2 Bartelmez G W 1953 Factors in the variability of the menstrual cycle Anat Rec 115 101 20
3 Berenblum I 1954 A speculative review the probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis Cancer Res 14 471 7
4 Burns R K Jr 1942 Hormones and the growth of the parts of the urogenital apparatus in mammalian embryos Cold Spring Harbor Symp Quant Biol 10 27-33
5 Cabot Case #34042 1948 New England J Med 238 130 32
6 Cabot Case #41461 1955 New England J Med 253 874 8
7 Corner G W Hartman C G and Bartelmez G W 1945 Development organization and breakdown of the corpus luteum in the rhesus monkey Carnegie Contrib Embryol No 204 117 46
8 Diddle A W 1952 Granulosa and theca cell ovarian tumors prognosis Cancer 5 215 28
9 Edmondson H A Levi L M Evans N and Horn P 1945 Papillary lesions of the cervix uteri in pregnancy Am J Obst & Gynec 49 356 7
10 Emge L A 1953 Endometrial cancer and feminizing tumors of the ovaries significance of their coexistence Obst & Gynec 1 511 15
11 Govan A D T 1954 Renal changes in eclampsia J Path & Bact 67 311 22
12 Grady H G 1954 Case No 3 in Fifteenth Conference on Surgical Pathology New York State Association of Public Health Laboratories pp 12 19
13 Greene R R and Peckham B 1954 Squamous papillomas of the cervix Am J Obst & Gynec 67 883 96
14 Gusberg S B 1947 Precursors of corpus carcinoma I Estrogens and adenomatous hyperplasia Am J Obst & Gynec 54 905 27
15 Gusberg S B Moore D B and Martin F 1954 Precursors of corpus carcinoma II A clinical and pathologic study of adenomatous hyperplasia Am J Obst & Gynec 68 1472 81
16 Hamperl H 1954 Über endometriale Granulocyten (endometriale kornchenzellen) Klin Wchnschr 29/30 665 8
17 Hellman L M Rosenthal A H Kistner R W and Gordon R 1954 Some factors influencing the proliferation of reserve cells in the human cervix Am J Obst & Gynec 67 899 911
18 Hellweg G 1954 Über endometriale Kornchenzellen (endometriale Granulocyten) Arch f Gynak 185 150 66
19 ——— 1955 Die endometrialen Kornchenzellen Geburtsh u Frauenheilk 6 521 30
20 Hertig A T and Edmonds H W 1940 Genesis of hydatidiform mole Arch Path 30 260 91
21 Hertig A T 1945 Diagnosing the endometrial biopsy Proc Conference on Diagnosis in Sterility ed by E T Engle
22 Hertig A T Sommers S C and Bengloff H 1949 Genesis of endometrial carcinoma III Carcinoma in situ Cancer 2 964 71
23 Hertig A T and Sheldon W H 1947 Hydatidiform mole—a pathologico-clinical correlation of 200 cases Am J Obst & Gynec 53 1 36
24 Hertig A T 1950 Hydatidiform mole and chorion epithelioma Progress in Gynecology vol II pp 372 94
25 Hertz R Cromer J K Young J P and Westfall B B 1951 Observations on the effect of progesterone on carcinoma of the cervix Symposium on Steroids in Clinical and Experimental Practice ed by A White Philadelphia The Blakiston Co pp 366 74
26 Iverson L 1947 Masculinizing tumors of the ovary S G & O 84 213 38
27 Javert C T and Finn W F 1951 Arrhenoblastoma the incidence of malignancy and the relationship to pregnancy sterility and to treatment Cancer 4 60 77
28 Jolly H Sexual Precocity Charles C Thomas Springfield Ill 1955
29 Jones H W and Jones G E S 1954 The gynecologic aspects of adrenal hyperplasia and allied disorders Am J Obst & Gynec 68 1330 65
30 Knobil E and Briggs F N 1955 Fetal maternal endocrine inter relations the hypophyseal adrenal system Endocrinol 57 147 52
31 Larson J A 1954 Estrogens and endometrial carcinoma Obst & Gynec 3 551 72
32 Lattes R Shepherd F Tovell H and Wylie R A clinical and pathologic study of endometriosis of the lung S G & O In press
33 Lipschutz A 1942 Induction and prevention of abdominal fibroids by steroid hormones and their bearing on growth and development Cold Spring Harbor Symp Quant Biol 10 79 90
34 Mandel W Evans E W and Walford R L 1954 Dissecting aortic aneurysm during pregnancy New England J Med 251 1059 61
35 Mansell H and Hertig A T 1955 Granulosa theca cell tumors and endometrial carcinoma Obst & Gynec 8 385 94
36 McKay D G and Robinson D 1947 Observations on fluorescence birefringence and histochemistry of the human ovary during the menstrual cycle Endocrinol 41 378 94
37 McKay D G Robinson D and Hertig A T 1949 Histochemical observations on granulosa cell tumors Am J Obst & Gynec 58 625 39
38 McKay D G Hertig A T and Hickey W F 1953 The histogenesis of granulosa and theca cell tumors of the human ovary Obst & Gynec 1 125-36
39 McKay D G Hertig A T Bardawil W A and Velardo J T 1956 Histochemical observations on the endometrium I Normal endometrium Obst & Gynec 8 22 39 II Abnormal endometrium 8 140 56
40 Nadel E M 1950 Histopathology of estrogen induced tumors in guinea pigs J Nat Cancer Inst 10 1043 65
41 Nelson H B Gillespie L and White P 1953 Pregnancy complicated by diabetes mellitus Obst & Gynec 1 219 25
42 Nelson W W and Greene R R 1953 The human ovary in pregnancy S G & O (Intl Abstr Surg) 97 1 22
43 Novak E and Rutledge F 1948 Atypical endometrial hyperplasia simulating adenocarcinoma Am J Obst & Gynec 55 46 53
44 Novak E R 1956 Postmenopausal endometrial hyperplasia Am J Obst & Gynec 71 1312 21
45 Noyes R W Hertig A T and Rock J 1950 Dating the endometrial biopsy Fertil & Steril 1 3 25

46 Park W W and Lees J C 1950 Choriocarcinoma Arch Path 49 73 104 205 41
47 Park W W 1954 The occurrence of decidual tissue within the lung report of a case J Path & Bact 67 563 70
48 Peckham B Greene R R Chung J T Bayly M A and Benaron H B W 1954 Epithelial abnormalities of the cervix during pregnancy Am J Obst & Gynec 67 21 31
49 —— 1954 The relation of pregnancy to abnormalities of the cervical epithelium S G & O 99 401 12
50 Romney S L Hertig A T and Reid D E 1950 The endometria associated with ectopic pregnancy S G & O 91 605 11
51 Schnitker M and Bayer C A 1944 Dissecting aneurysms in young individual particularly in association with pregnancy with report of a case Ann Int Med 20 486 511
52 Shaw W 1927 Some pathologic forms of corpus luteum J Obst & Gynaec Brit Emp 34 300 30 1927
53 Smith G van S 1947 Menstrual toxin II Clinical significance Am J Obst & Gynec 54 212 18
54 Smith G van S and Smith O W 1934 Excessive gonad stimulating hormone and subnormal amounts of eostrin in the toxaemias of late pregnancy Am J Physiol 107 128 45
55 —— 1948 Internal secretions and toxemia of late pregnancy Physiol Rev 28 1 22
56 Smith O W 1947 Menstrual toxin I Experimental studies Am J Obst & Gynec 54 201 11
57 Speert H 1948 Corpus cancer clinical pathological and etiological aspects Cancer 1 584 603
58 Stein I F and Leventhal M L 1935 Amenorrhea associated with bilateral polycystic ovaries Am J Obst & Gynec 29 181 91
59 Sternberg W H 1949 Morphology androgenic function hyperplasia and tumors of the human ovarian hilus cell Am J Path 25 493 521
60 Symeonidis A 1949 Eclampsia like condition in pregnant rats injected with progesterone J Nat Cancer Inst 10 711 17
61 Tannhauser S 1951 Myometrium and leiomyomas in endometrial carcinoma Failure of involution and reactivation of smooth muscle Am J Obst & Gynec 61 265 75 1951
62 Teilum G 1946 Arrhenoblastoma—androblastoma Acta Path et Microbiol Scandinav 23 252 64
63 —— 1951 Classification of ovarian tumors Acta Obst et Gynec Scandinav 31 292 312
64 Turner S J 1943 Bilateral microcystic degeneration of the ovaries and masculinizing syndrome Am J Obst & Gynec 46 295 8
65 Weaver J D 1950 Nongonorrheal vulvovaginitis due to gram negative intracellular diplococci Am J Obst & Gynec 60 257 9
66 White R F Hertig A T Rock J and Adams E 1951 Histological and histochemical observations on the corpus luteum of human pregnancy Carnegie Contrib to Embryol No 224 55 74
67 Woll E Hertig A T Smith G Van S and Johnson L C 1948 The ovary in endometrial carcinoma Am J Obst & Gynec 56 617 33
68 Morris J McL and Scully R E 1958 Endocrine Pathology of the Ovary 151 pp St Louis C V Mosby Co

X

Endocrine Pathology of the Male Reproductive System

ROBERT H FENNELL JR M D

THE AGENTS that produce injury as well as the responses of tissues to such injury are fundamentally the same in the reproductive system of the male as in other organ systems of the body The pathologic anatomy of the male reproductive system therefore presents few unique problems For this reason the basic principles of pathologic anatomy will be left to the standard textbooks as well as much of the routine descriptive anatomy of the more common diseases Emphasis in this chapter will be placed upon changes that are recognized as being related to the endocrine glands although it is sometimes difficult to delimit what is or is not related to hormones Furthermore material will be specifically oriented to aid the physician studying the infertile male Descriptive anatomy of some diseases frequently seen by the physician who examines the infertile male will be included although many such diseases have no direct relation to endocrine disorders

TESTIS

Anomalies

Cryptorchidism is the most important and most common of the maldevelopments that affect the testis The incidence varies in different age groups Ten per cent of newborn males have undescended testes 2 per cent of prepuberal boys and 0 1 per cent to 0 3 per cent of adults are so affected The process may therefore be thought of as one that is continuous until the onset of puberty However the decrease in prevalence from birth to puberty points to the frequency with which the cryptorchid testis spontaneously finds its normal position Cases of cryptorchidism that persist to puberty are often remediable

The ectopic testis may be found in different places along its expected route of descent to the scrotum It may be beyond this route and be found in the superficial tissues of the thigh or in the perineum Most commonly the testis is in the inguinal canal but cannot be moved into the scrotum Gross [9] found nondescent more often on the right side (45%) than on the left (30%) and in 25 per cent of his patients both testes were undescended An indirect inguinal hernia is almost always associated with cryptorchidism

There are several factors that make cryptorchidism important to recognize and to remedy The undescended testis lacks the heat regulation normally afforded by the scrotum and the higher intra abdominal temperature results in testicular damage [70] There is a greater incidence of neoplasia in the retained testis Twenty four of the 990 testicular tumors in the files of the Armed Forces Institute of Pathology occurred in undescended testes This represents an incidence of tumor formation in men with cryptorchidism that is ten times greater than the incidence in normally situated gonads [21] Also when located in the inguinal canal the testis is more susceptible to trauma

Until early puberty there is no perceptible difference between a retained testis and one in the scrotum In early puberty the tubules of the scrotal testis begin to enlarge and germ cells to grow In the undescended testis however the immature tubules persist unchanged As the scrotal testis develops into a mature gonad the difference between it and the cryptorchid testis becomes progressively more evident Variation in the degree of abnormality may occur in different parts of a retained testis and the histology of undescended gonads from men of the same age is not uniform However damage tends to be worse with increasing age

Grossly the retained gonad is smaller and the tunica is wrinkled Histologically there is retarded spermatogenesis Tubules contain spermatogonia and spermatids but with more advanced atrophy only Sertoli cells line the tubules The tubular walls are thickened and hyalinized and may become lumenless fibrous cords (Fig 1) Leydig cells are conspicuous but this is relative and probably no increase occurs They appear normal or are sometimes vacuolated Fibrosis of interstitial tissue may be severe by middle age in the undescended testis

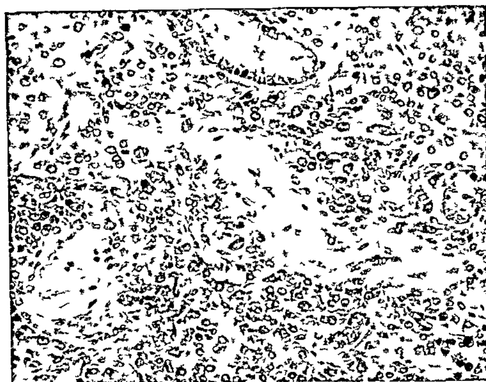


FIG 1 CRYPTORCHID TESTIS FROM MIDDLE AGED MAN The tubules are cords of hyalinized connective tissue surrounded by masses of Leydig cells × 300 (Courtesy of Dr E R Fisher)

Though eunuchoidism rarely develops reduced androgen excretion in men with cryptorchidism has been reported

Immature tubules may persist in the ectopic testis [3] as clusters of microscopic size The undifferentiated cells that line these tubules are benign though they have large nuclei On the basis of these nuclei such tubules have been mistakenly called low grade adenocarcinomas [82]

The idea has been enunciated that the undescended testis is intrinsically an abnormal organ and that it is incapable of normal development [5] Enthusiastic supporters of orchiopexy cite results of surgery [29] that contradict this idea A more moderate viewpoint is that an undescended testis may be intrinsically abnormal in some cases while in other cases except for its ectopy the testis may be normal If it is an abnormal organ attempts to produce a gonad capable of spermatogenesis will be futile [80] but if ectopy is the only fault therapy can be successful

Related to the divergent opinions about the inherent potential for spermatogenesis in the undescended gonad are differing opinions about the value of orchiopexy In a report of 1222 operations for cryptorchidism there were 38 patients for whom there was follow up data adequate to evaluate fertility Thirty (79%) of the 38 patients were fertile as evidenced by production of offspring or by normal sperm count [29] Despite such results there is skepticism about favorable effects of orchiopexy [111] Damage to the testis by the trauma of surgery is a factor to be considered protagonists of surgery [4] however insist that careful technique minimizes trauma Little or no value is attributed to orchiopexy in preventing malignancy but it is argued that tumor formation in the scrotal testis is more easily detected and treated than if the organ is in the abdomen A psychological value is also cited as a reason for surgical placement of the testis in the scrotum

Experimentally produced cryptorchidism is severely damaging In a week or less tubules of the adult guinea pig show derangement and dissolution Desquamation and disintegration of cells occur and giant cells form Later the tubules are shrunken and lined by a layer of Sertoli cells Leydig cells appear concentrated between the shrunken tubules When the testis is returned to the scrotum after three weeks in the abdomen a few tubules remain abnormal but spermatogenesis soon becomes normal in most instances Testes of the newborn guinea pig if fixed in the abdomen remain immature If placed in the scrotum after three months in the abdomen they promptly assume spermatogenesis

Several practical facts have emerged from studies of the cryptorchid testis The decrease in incidence of cryptorchidism from birth to puberty points to the

frequency with which the cryptorchid testis spontaneously finds its normal position In cases where the testis remains undescended a corrective procedure should be done by the time of puberty or quite soon thereafter if a testis capable of spermatogenesis is to be expected A biopsy done at the time of surgery will indicate whether the testis is capable of normal development Much more can be learned by the study of biopsies It may be possible to determine which testes should be removed rather than transplanted

Other anomalies of the testis are rare Varying degrees of malformation or even complete absence may occur As noted below an absence of germ cells is thought to be a congenital defect

Infections

As compared with the epididymis or prostate the testis is relatively immune to infection Diseases that become widespread such as bacterial endocarditis may involve the testis but this is unusual Infectious diseases that involve other parts of the body do produce damage to the testis[69] but the effect is probably related primarily to the fever resulting from the infection

Syphilitic orchitis is now quite an uncommon disease It usually occurs in the tertiary stage of syphilis as a diffuse scarring or a gumma The latter is a circumscribed rubbery gray nodule which microscopically shows necrosis but with some preservation of architecture Around the necrotic area are lymphocytes plasma cells and multinucleated giant cells Peripherally the blood vessels have a ring of inflammatory cells about them The *Treponema pallidum* may be found with great difficulty

When *tuberculosis* involves the testis it is most often secondary to tuberculous epididymitis

Mumps orchitis occurs often enough for it to be considered as the possible cause in any case of male infertility The incidence of mumps orchitis varies in different series from as low as 11 per cent to as high as 36 per cent With rare exceptions it develops only in postpuberal males Among 2000 healthy men being discharged from the Army 1086 (54 3%) gave a history of having had mumps[108] Only 4 9 per cent of the 1086 had orchitis but in the group that had mumps after the age of 13 years 19 per cent had orchitis Atrophy of one or both testicles was found in 35 8 per cent of those with a history of orchitis In most series of mumps orchitis atrophy is reported in approximately 50 per cent Orchitis is bilateral in one third of the cases but bilateral atrophy is less common It occurred in only 10 per cent of Werner s series[108]

To evaluate mumps orchitis as a cause of sterility Werner[109] examined the seminal fluid of 49 males who gave a history of mumps orchitis Only one of these men had azoospermia but fertility was considered as impaired in 13 per cent of the men with orchitis when compared to the 91 normal controls Case findings in an infertility clinic give added importance to mumps orchitis Of 19 men who came to the clinic complaining of infertility and who had a history of mumps orchitis 9 were azoospermic and 6 had a reduced sperm count[6] Impairment of endocrine function of the testis manifested by eunuchoidism is so rare following mumps orchitis that it must be considered as probably coincidental

An epidemic of mumps among military personnel at a time when orchidotomy was practiced to relieve intratesticular pressure has furnished a series of biopsies of acute orchitis[4] all of which were obtained within 5 days of onset of symptoms of orchitis A sequence was thought to be evident starting with early edema and scant lymphocytic reaction The lymphocytic infiltrate is later diffuse with focal hemorrhages Germinal epithelium is destroyed and tubules are filled with fibrin neutrophilic leukocytes and cellular debris In these small biopsies patchiness of tubular involvement is evident though some tubules are diffusely damaged Infiltration of interstitial tissue with inflammatory exudate is marked in the more severe and presumably later stages An autopsied case in which death occurred at 11 days had areas of thickened lamina propria with scarring and disappearance of the Sertoli cells Such areas were considered as probable sites of permanent damage and as the explanation for the islands of hyalinized tubules found at necropsy in testicles of patients dying of unrelated conditions (Fig 2) The patchy nature of damage suggests that there should be no clinically significant effect A detailed description of an atrophied testis following mumps orchitis is given in an autopsy report by Hall[31] Tubules are seen that have completely lost their epithelium and been converted to fibrous cords but less severely damaged tubules are also present Interstitial changes are not pronounced Inflammatory cells are present diffusely and in clusters Unaffected areas appear completely normal with active spermatogenesis

Effects of Various Noxious Agents

Radiation The germinal epithelium of the testis is one of the most sensitive tissues of the body to ionizing radiation The sterilizing effect of radiation became known early in its history Sterility can be produced by a single large dose or repeated small doses but there is considerable individual variation in the dose of radiation required to produce sterility Ionizing radiation from various sources apparently has no distinctive effects except for the variation in

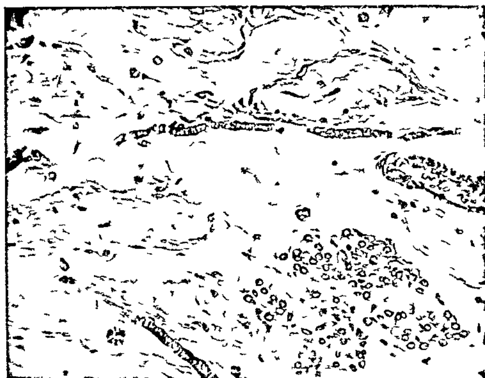


FIG 2 A FOCUS OF SCARRED TUBULES IN TESTIS Such scars may result from mumps orchitis × 300

penetrability i e a source of beta rays at the surface of the testicle would produce damage to the superficial tubules only

Within several days after exposure to a large dose of radiation severe damage is evident microscopically (Fig 3) Mitoses are decreased and necrotic cells lie free in the tubules Mature sperm and spermatogonia are less severely damaged Sertoli cells are relatively resistant and Leydig cells are unaltered by doses that destroy the germinal epithelium The resistance of the mature sperm is reflected in the presence of live sperm in the ejaculate which gradually disappear after several weeks as no new ones are formed Giant cell forms seen in tubular lumens are thought to result from fusion of spermatids Several months after an exposure to a large dose of radiation Sertoli cells are prominent and a few bizarre spermatogonia are present in the tubules Leydig cells are conspicuous perhaps due to tubular shrinkage Later basement membranes are thickened and tubules are smaller Persistence of Leydig cells is reflected in the continuation of sexual function The testis may be smaller and soft but potency is maintained and there is no loss of male secondary sex characters

Reports of accidents with atomic reactors have re-emphasized the variableness of individual susceptibility to radiation [41] A quantity of radiation ordinarily considered to be a sterilizing dose has not had this effect on some individuals One report [8] includes observation of the testis by biopsy during recovery Spermatogenesis began to return 12 months after exposure to radiation though many tubules remained scarred

Trauma Inguinal herniorrhaphy is recognized as one of the causes of testicular atrophy A survey of 10 000 inductees revealed 152 instances of testicular atrophy of these 11 were thought to be due to a previous herniorrhaphy Five of the 11 men had atrophy that was so severe as to be classed as almost complete A follow up of patients operated upon for inguinal hernia showed 6 7 per cent to have partial atrophy of the testis Pain and swelling of the scrotum following herniorrhaphy may be warning signals that atrophy will follow and immediate intervention is indicated [4] Operation for varicocele or hydrocele or any other procedure that necessitates handling of the testis may be damaging

Impairment of blood supply to the testis is probably the actual cause of atrophy following surgical procedures In dogs it has been shown experimentally that 1 or 2 hours of ischemia produces irreversible damage [13] Impairment of nerve supply has been suggested as the explanation of testicular damage in paraplegics [9] The same explanation might be given for postoperative atrophy but there is little evidence to support it

Torsion of the spermatic cord is often considered



FIG 3 TESTIS OF GUINEA PIG 10 DAYS AFTER IRRADIATION Degenerating and necrotic cells are numerous and giant cells have formed Interstitial cells are unaltered × 300

as traumatic in origin although no history of trauma is usually elicited Torsion of the cord occurs against a background of developmental abnormality About half of the reported examples of torsion of the cord involve incompletely descended testes[19] An abnormality of the tunica permits torsion to occur when the testis is in the scrotum The abnormality that permits twisting of the cord is an extension of the tunica vaginalis over the entire testis epididymis and lower cord The testis instead of being fixed to one side by parietal tunica vaginalis is suspended free in the sac The torsion of the cord causes obstruction of venous and later arterial blood Infarction of the testis soon develops Spontaneous reduction of the torsion can happen but repeated episodes may result in testicular atrophy Seven per cent of torsions are bilateral

Heat Lack of proper heat regulation was mentioned earlier as one of the causes of damage to undescended testes The higher temperature inside the body was thought to be the cause of depressed spermatogenesis in experimental cryptorchidism[70] Exposure of the testis to an elevated temperature by placing the animal in an environment of high temperatures resulted in diminished spermatogenesis thus confirming the idea that heat is the causal factor[70] In healthy human males exposure to temperatures of 41 C for several hours resulted in a decline in the sperm count which became apparent in three weeks The mean low level was reached in 42 days and the return to normal was gradual[6] Fever due to infections has been reported as a cause of depression of sperm counts Two cases of chicken pox and one of pneumonia all with fever were followed by reduced sperm counts with only a few motile forms in the semen[61]

Appreciation of the importance of proper heat regulation by the scrotum has resulted in particular attention to the clothing of men with fertility problems Simmons[90] found significant elevation of sperm counts (and resulting pregnancies) after discarding of the skin tight or Jockey type of underwear The effect of working about foundries or bakeries where high environmental temperatures occur must be considered in men with sterility problems

Malnutrition It is difficult to evaluate malnutrition as a damaging factor to the testis because malnutrition in this country is usually secondary to an infectious or neoplastic process and these processes complicate interpretation of changes Specific deficiencies have been produced in animals e g vitamin E deficiency results in degeneration of tubular epithelium and ultimately in aspermatogenesis[85] Other deficiencies including various mineral vitamin and protein deficiencies damage germinal epithelium in animals Rats that are underfed become sterile be

cause of atrophy of accessory genital organs That chorionic gonadotropins will restore function despite starvation indicates damage to the pituitary

No specific effect of dietary deficiencies including vitamin E have been described in humans Undoubtedly severe and prolonged malnutrition produces changes in the testis In American soldiers released from prisoner of war camps the testes were atrophic and the tubules were hyalinized but Leydig cells were preserved[53] Other prisoners of war were studied a few weeks after release from prison and normal semen specimens were found In the Minnesota experiment where a state of semi starvation was produced little effect on volume of ejaculate and sperm count was found but motility of sperm was reduced

Disorders of Testis Related to Endocrines

The use of the testicular biopsy has contributed significantly to knowledge of anatomy and physiology of the testis in the past 15 years Biopsies of the testis can be made more easily and yield more information than those of the endometrium Both gametogenic cells and hormone producing cells can be evaluated by testicular biopsy The procedure is harmless except for the occurrence of a rare hematoma Biopsies of bull testes cause transient depression of spermatogenesis but complete recovery follows[2]

Histologic studies of the testis have stimulated interest in its endocrine functions and its relation to the pituitary A concept that has been widely accepted is that the pituitary gives rise to two hormones a follicle stimulating hormone (FSH) and an interstitial cell stimulating hormone (ICSH) FSH acts upon tubules of the testis to stimulate spermatogenesis and ICSH acts upon the interstitial cells which elaborate testosterone The principal point on which disagreement occurs is whether or not a hormone is formed by the tubules Howard *et al*[46] present diagramatically the concept that FSH stimulates Sertoli cells in the tubules to form an x hormone which with the testosterone from the Leydig cells stimulates spermatogenesis The anatomic alterations that support their argument for a hormone from the tubules is the presence of high FSH in conditions of tubular destruction with intact Leydig cells If Leydig cells were the only source of hormone in the testis FSH should not be elevated when these cells are intact There are others[6] who agree that a hormone comes from the seminiferous tubules and cite the same evidence as given above plus the evidence that aqueous extracts of bull testis prevent castration hypertrophy in the pituitary Opponents of the idea of testicular hormone in addition to testosterone advance the evidence that gonadotropin titers are elevated in conditions in which the Sertoli cells are preserved and in germinal cell arrest[40] Other arguments hinging on anatomical changes concern the question of whether Leydig cells form estrogens[61] The evidence is not convincing that they do and in animals it is the Sertoli cell tumors that produce estrogens

Many detailed studies[1, 3, 46] have correlated sperm count histology of the testis and endocrine status of the patient However no satisfactory classification of endocrine disorders affecting the testis has evolved The larger series have depended upon the level of gonadotropins in classifying diseases of the testis One group[38] uses the term gonadotropins which includes FSH ICSH and perhaps other unknown substances that stimulate or inhibit the testis Another group speaks in their classification simply of the FSH[4] Albert *et al*[1] offer a grouping of testicular disorders based entirely on anatomic criteria but this is cumbersome and difficult to use In this discussion the widely accepted system based on gonadotropin level will be used

Testicular Diseases with Low Gonadotropins Low follicle stimulating hormone or hypogonadotropic disorders are attributed to malfunction of the pituitary Often no abnormality of the pituitary other than testicular dysfunction is demonstrable by any means in almost half of some series craniopharyngioma suprasellar cyst or other recognized pituitary lesions are present These latter patients may also have manifestations of some degree of panhypopituitarism If the pituitary is damaged before puberty the testis is simply immature Tubules exist as lumenless cords and the Sertoli cells are undifferentiated Slight mitotic activity is seen in germinal cells and there are no Leydig cells

There is a different histologic picture if the testis has been stimulated and the stimulus later withdrawn In this case the tunica propria is thickened often quite markedly Germ cell activity is impaired and there is peritubular fibrosis and Leydig cell degeneration Accumulations of lipoid material are seen in Sertoli cells these cells sometimes filling the tubular lumens

Testicular Diseases with High Gonadotropins If the level of gonadotropins is high the defect is assumed to be in the testis The testicular lesion may be of congenital traumatic infectious or of unknown etiology the last group being quite large An elevated FSH usually but not always is present in conditions in which no germ cells are found This defect of germ cells is considered by some to be congenital Other patients with the same histologic change have given a history of exposure to radiation The tubules are of nearly normal size but no germ cells are present The Sertoli cells that line the tubules seem quite normal The tubules are not thickened and

there is no fibrosis. Leydig cells appear normal though increased in number.

The most frequently encountered condition in this subdivision is one described by Klinefelter *et al.* (Klinefelter's syndrome).[54] It is characterized by gynecomastia, aspermatogenesis without a Leydigism and increased excretion of follicle stimulating hormone. Others[37] emphasize that presence or absence of gynecomastia is not a fundamental differentiating point. The testes are usually small and firm and the histology is that of sclerosing tubular degeneration with prominence of Leydig cells (Figs. 4 and 5). The process progresses from the reduction of spermatogenesis, atrophy of Sertoli cells and thickening of the tunica propria to disappearance of germ cells and Sertoli cells with the thickened tunica propria filling the lumen. Sniffen[95] in a lucid description of the changes emphasizes the inverse relation between Sertoli cells and tunica propria ingrowth, atrophy of the former being accompanied by a thickened tunica in any given tubule. He considers the thickening to be of the tunica propria, not of the basement membrane since the latter can be seen as a thin membrane after the deposition of collagen. The Leydig cells are very conspicuous and are probably hyperplastic. They are normal in appearance in some cases (Fig. 5), but are often abnormal in various degrees. The cells may be irregular in shape, have indistinct borders and pyknotic nuclei; in such cells there are usually no crystalloids or pigment. Cells with giant nuclei are sometimes encountered.

The breasts that are removed, usually for cosmetic reasons, from patients with Klinefelter's syndrome show histologically increased interlobular connective tissue that is quite dense and abundant. No formation of lobules occurs. There is no active duct growth such as is seen in men who receive estrogens.

An autopsy report[10] of a patient who had Klinefelter's syndrome and a review of other cases of suspected Klinefelter's syndrome suggest it to be a multiglandular disorder. The thyroid and adrenal were hyperplastic in several cases. The pituitary in the reported case contained a high percentage of amphophils which may be the source of elevated urinary gonadotropin.

As a result of a clinical and histologic study,[17] one hypothesis concerning Klinefelter's syndrome is that at puberty there occurs a disturbance in development of Leydig cells presumably due to luteinizing hormone deficiency or inability of Leydig cells to respond. The resultant damage to intertubular connective tissue and to the fibroblasts of the walls of the tubules causes thickening of the walls, which in turn causes nutritional damage to the tubules.

An interesting finding with reference to the nature of Klinefelter's syndrome is the description of nuclear chromatin masses characteristically found in females in the somatic cells of several patients with this syndrome.[9, 83] In both of the cited reports testicular tissue was identified in the individuals under consideration. The studies of chromosomal sex in Turner's syndrome that indicate a male pattern and the explanation[30] that early harmful influences are the cause may give a new lead for investigation of Klinefelter's syndrome.

There is no clear cut climacteric in the male as there is in the female, and the gonadotropins are not usually elevated at this age. A small series of patients has been studied who presented symptoms similar to the menopause in women and in whom the gonadotropins were elevated. Biopsies of the testes in these subjects have been described as showing reduction in size and activity of tubules and Leydig cells.[79] Others[95] have found no deviation from normal in testicular biopsies of such patients.

A rare finding is complete absence of the testes. This is usually a developmental abnormality but can be of infectious or traumatic origin. The gonadotropins are high and the patients have eunuchoid proportions, high pitched voices and small prostates.

Testicular Diseases with Normal Gonadotropins. Certain men seeking help for sterility present an interesting problem. These men have no abnormality of the gonadotropins and are of normal male habitus. The testes are within the normal range of size and consistency and histologically the only abnormality is an arrest of maturation of spermatogenesis. The most commonly encountered point of arrest is at the primary spermatocyte level. Arrest occurs at different stages, however, and spermatids are seen as the point of arrest in some. Usually in a given testis maturation ceases at the same cell level. The tubules have a thin basement membrane and Sertoli cells are normal as are the spermatogonia. The Leydig cells are in normal numbers and are of normal structure.

Related perhaps to the group above in that there are no detectable endocrine abnormalities are men who present as sterility problems and in whom the biopsy shows reduction in spermatogenesis. There is no uniform point of arrest, for maturation does occur but the number of any cell type is reduced and the severity may be quite variable in different testes. In the more severe examples few germ cells are seen and Sertoli cells line much of the tubule. No sclerosis of the tubule is seen and the Leydig cells appear normal.

In attempting to explain the cause of arrested maturation and hypospermatogenesis Howard *et al.*[46] have considered various factors but have dismissed the possibility of endocrinologic origin. Nutritional deficiency is considered as one possibility. Sniffen *et al.*[94] compare the effect of benzol on bone marrow with the picture seen in arrested maturation in the testis.



FIG 4 SCLEROSING TUBULAR DEGENERATION (Klinefelter's syndrome) Masses of Leydig cells surround partially hyalinized tubules × 300 (Courtesy of Dr R C Sniffen)

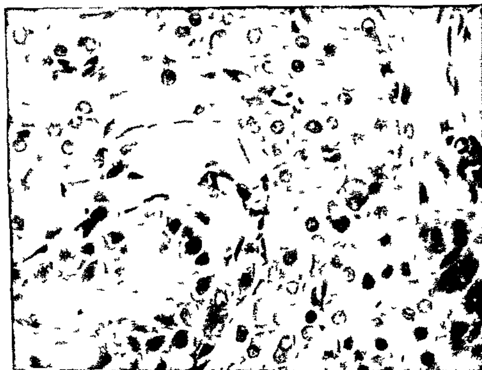


FIG 5 SCLEROSING TUBULAR DEGENERATION (Klinefelter's syndrome) A higher magnification of Figure 6 shows Leydig cells about a thickened tubule containing atrophic Sertoli cells × 600 (Courtesy of Dr R. C Sniffen)

there is no fibrosis Leydig cells appear normal though increased in number

The most frequently encountered condition in this subdivision is one described by Klinefelter *et al* (Klinefelter's syndrome)[54] It is characterized by gynecomastia aspermatogenesis without a Leydigism and increased excretion of follicle stimulating hormone Others[37] emphasize that presence or absence of gynecomastia is not a fundamental differentiating point The testes are usually small and firm and the histology is that of sclerosing tubular degeneration with prominence of Leydig cells (Figs 4 and 5) The process progresses from the reduction of spermatogenesis atrophy of Sertoli cells and thickening of the tunica propria to disappearance of germ cells and Sertoli cells with the thickened tunica propria filling the lumen Sniffen[95] in a lucid description of the changes emphasizes the inverse relation between Sertoli cells and tunica propria ingrowth atrophy of the former being accompanied by a thickened tunica in any given tubule He considers the thickening to be of the tunica propria not of the basement membrane since the latter can be seen as a thin membrane after the deposition of collagen The Leydig cells are very conspicuous and are probably hyperplastic They are normal in appearance in some cases (Fig 5) but are often abnormal in various degrees The cells may be irregular in shape have indistinct borders and pyknotic nuclei in such cells there are usually no crystalloids or pigment Cells with giant nuclei are sometimes encountered

The breasts that are removed usually for cosmetic reasons from patients with Klinefelter's syndrome show histologically increased interlobular connective tissue that is quite dense and abundant No formation of lobules occurs There is no active duct growth such as is seen in men who receive estrogens

An autopsy report[10] of a patient who had Klinefelter's syndrome and a review of other cases of suspected Klinefelter's syndrome suggest it to be a multiglandular disorder The thyroid and adrenal were hyperplastic in several cases The pituitary in the reported case contained a high percentage of amphophils which may be the source of elevated urinary gonadotropin

As a result of a clinical and histologic study[17] one hypothesis concerning Klinefelter's syndrome is that at puberty there occurs a disturbance in development of Leydig cells presumably due to luteinizing hormone deficiency or inability of Leydig cells to respond *The resultant* damage to intertubular connective tissue and to the fibroblasts of the walls of the tubules causes thickening of the walls which in turn causes nutritional damage to the tubules

An interesting finding with reference to the nature of Klinefelter's syndrome is the description of nuclear chromatin masses characteristically found in females in the somatic cells of several patients with this syndrome[9, 83] In both of the cited reports testicular tissue was identified in the individuals under consideration The studies of chromosomal sex in Turner's syndrome that indicate a male pattern and the explanation[80] that early harmful influences are the cause may give a new lead for investigation of Klinefelter's syndrome

There is no clear cut climacteric in the male as there is in the female and the gonadotropins are not usually elevated at this age A small series of patients has been studied who presented symptoms similar to the menopause in women and in whom the gonadotropins were elevated Biopsies of the testes in these subjects have been described as showing reduction in size and activity of tubules and Leydig cells[79] Others[85] have found no deviation from normal in testicular biopsies of such patients

A rare finding is complete absence of the testes This is usually a developmental abnormality but can be of infectious or traumatic origin The gonadotropins are high and the patients have eunuchoid proportions high pitched voices and small prostates

Testicular Diseases with Normal Gonadotropins Certain men seeking help for sterility present an interesting problem These men have no abnormality of the gonadotropins and are of normal male habitus The testes are within the normal range of size and consistency and histologically the only abnormality is an arrest of maturation of spermatogenesis The most commonly encountered point of arrest is at the primary spermatocyte level Arrest occurs at different stages however and spermatids are seen as the point of arrest in some Usually in a given testis maturation ceases at the same cell level The tubules have a thin basement membrane and *Sertoli cells* are normal as are the spermatogonia The Leydig cells are in normal numbers and are of normal structure

Related perhaps to the group above in that there are no detectable endocrine abnormalities are men who present as sterility problems and in whom the biopsy shows reduction in spermatogenesis There is no uniform point of arrest for maturation does occur but the number of any cell type is reduced and the severity may be quite variable in different testes In the more severe examples few germ cells are seen and Sertoli cells line much of the tubule No sclerosis of the tubule is seen and the Leydig cells appear normal

In attempting to explain the cause of arrested maturation and hypospermatogenesis Howard *et al*[46] have considered various factors but have dismissed the possibility of endocrinologic origin Nutritional deficiency is considered as one possibility Sniffen *et al*[94] compare the effect of benzol on bone marrow with the picture seen in arrested maturation in the testis

mechanism of tubular damage is thought to be secondary to suppression of pituitary FSH formation The pituitary is suppressed by the higher levels of estrogens and androgens both shown to be present during administration of chorionic gonadotropin

Precocious Puberty

Testicular biopsy has been recommended to distinguish the types of precocious puberty [97] In true precocious puberty interstitial cells are present and the tubules show varying degrees of spermatogenic activity Interstitial cells are not present in adrenal virilism and spermatogenic activity does not usually occur It should be remembered that with adrenal virilism cells are sometimes present in the testis that resemble interstitial cells Spermatogenic activity has also been described [85] with adrenal virilism but this is quite rare

Histochemistry of Testicular Diseases

Histochemical and cytochemical studies of the testis have added to an understanding of the functioning of different cell types As expected Leydig cells and Sertoli cells have lipids probably ketosteroids [3] Suggestive abnormalities of Sertoli cells have been found in sterility patients [58] Other studies have been applied to specific types of abnormalities [19]

Leuchtenberger *et al* [55] used microspectrophotometric methods to measure the content of desoxyribose nuclei acid (DNA) in the spermatozoa of fertile and infertile men The ratio of 4 2 1 is maintained for the DNA content of primary spermatocytes secondary spermatocytes and spermatids respectively whether the men were fertile or infertile The infertile men however have a consistently lower DNA content The sperm are otherwise morphologically normal and there is active spermatogenesis The ability of a sperm to fertilize therefore seems to be linked with its DNA content

Tumors

Figures based on histologically proven cases show the incidence of testicular tumors to be 2 88 per 100 000 per year in the Army population [0] A higher incidence would be found in the civilian population because in this group there are more men in the fourth decade the period when the incidence of testicular tumors is highest Tumors of the testis comprise 1 5 per cent to 2 0 per cent of all cancers of the male Classification of testicular tumors has varied greatly The largest series of case is that re

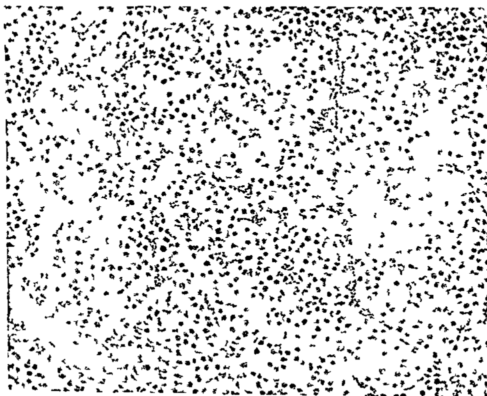


FIG 6 SEMINOMA OF TESTIS The stroma is scant contain few lymphocytes and the tumor cells are rather uniform X 185 (A F I P #100810) (Courtesy of Dr Frank J Dixon)

Of the various conditions that have been encountered in patients subjected to testicular biopsy the most important to recognize is obstruction of the excretory ducts Such obstruction is common and may be remediable Hormones have no effect There are no consistent features in the testicular biopsy that enable easy recognition of duct obstruction Sniffen[94] however describes desquamation of germ cells into the lumens This is sufficiently reliable for obstruction to be suspected from the histologic study of the biopsy[92] Many spermatocytes spermatids and spermatozoa are lying in the tubules and some are disintegrating Pyknotic nuclear fragments are evident Normal spermatogenesis is the general picture

Effects of Estrogen on the Testis Estrogen affects the testis through suppression of gonadotropin formation by the pituitary The effects of estrogen administration as seen in testicular morphology therefore are similar to those resulting from pituitary inhibition[96] Within several days of the beginning of diethylstilbestrol administration there is reduction of spermatogenic activity and accumulation of lipoid vacuoles in the cytoplasm of Sertoli cells The lamina propria of the tubules gradually becomes thickened with collagen and Leydig cells disappear or become smaller and densely pigmented After 2 weeks no sperm cells are formed and only a few early gametogenic cells remain Later shrunken tubules are lined by foamy Sertoli cells with only spermatogonia remaining The lamina propria is thickened and elastic fibers surround the tubules The few Leydig cells that persist are very abnormal being small vacuolated or disintegrating

Evidence that the damage is reversible is adduced by Sniffen *et al*[96] in a patient undergoing orchiectomy four months after taking large amounts of diethylstilbestrol for treatment of prostatic carcinoma In the orchiectomy specimen the Leydig cells appeared normal and tubules showed gradations from complete sclerosis to normal The testes studied by most observers for the effect of estrogens are abnormal as they are taken from elderly males with carcinoma of the prostate[1] Charny's patient[11] is an exception in that he was a young healthy male In this case the histologic picture is similar to that described by Sniffen

The effect of estrogens on the rat testis[60] is different from the effect on humans Tubular damage with persistence of spermatogonia and recovery after drug stoppage resemble the changes in the human testis but Leydig cells are unaltered In the mouse Leydig cell proliferation follows estrogen administration and certain resulting tumors are estrogen dependent

Damage to the testis in cirrhosis of the liver is ascribed to increased circulation of estrogens due to the failure of the malfunctioning liver to remove these substances Such a chronic debilitating disease often associated with fever can in itself be damaging but there seems to be an added factor of estrogen excess Testicular atrophy is described in 57 per cent[4] to 70 per cent[5] of cirrhotics by both clinical and microscopic criteria Gynecomastia has been long recognized as a frequent finding with advanced cirrhosis and altered estrogen excretion has been described[8] Experimentally animals in which the livers are damaged by carbon tetrachloride are very susceptible to testicular damage by estrogens[4] Spider telangiectases clinically characteristic of cirrhosis can be produced experimentally with estrogens[5] Endocrine changes other than an increase of estrogens may occur with cirrhosis Decreased axillary hair indicates diminished adrenocortical function

Digitalis is reported as an occasional cause of gynecomastia This is attributed to an estrogen like effect in view of the similar chemical configuration of the two No description of the effect of digitalis preparations on the testis is known to the writer

Effect of Testosterone on the Testis The mechanism of the action of testosterone is thought to be suppression of pituitary formation of gonadotropins Biopsies of the testis taken during administration of the drug have shown as might be expected changes similar to those seen with estrogen There is a decrease in size of the seminiferous tubules as well as sclerosis and hyalinization of their basement membrane and tunica propria Necrosis and sloughing of germinal elements are also seen Sperm formation is arrested and cells decrease in number with spermatids and spermatocytes disappearing more readily than spermatogonia Damage to Leydig cells is evidenced by absence of Leydig cells and atrophy of those interstitial cells that can be detected[39] The process appears to be reversible indeed the rebound that follows cessation of testosterone has resulted in optimism concerning the use of the drug for certain sterility problems[35] Few post treatment biopsies are available but the histology corresponds to the improved sperm count The return to better than pretreatment appearance is impressive in that hyalinized shrunken tubules are changed and Leydig cells reformed A note of caution however has been introduced concerning the use of testosterone for sterility[9]

Effect of Chorionic Gonadotropin on the Testis Chorionic gonadotropin has been shown[61] to have a damaging effect on tubules though not on the Leydig cells Tubules become smaller spermatogenesis ceases and germinal cells slough the basement membrane becomes hyalinized and there develops peritubular fibrosis The Leydig cells increase in number and contain more lipid droplets The

ported by the Armed Forces Institute of Pathology whose classification will be used here [9]

Germinal tumors arise from the germ cells and comprise 96 5 per cent of the 990 tumors reported by the A F I P The other 3 5 per cent is made up of non germinal tumors and includes interstitial cell tumors androblastomas and connective tissue tumors Metastatic tumors to the testis are so rare that they are not considered

Germinal Tumors Four basic histologic types of germinal cell tumors are recognized seminoma embryonal carcinoma teratoma and choriocarcinoma The seminomas are composed of large round or polygonal cells with distinct borders and clear cytoplasm (Fig 6) The large nucleus is moderately hyperchromatic usually containing distinct nucleoli (Fig 7) The stroma of the seminoma may be predominantly lymphoid tissue and a granulomatous reaction is common Patches of ischemic necrosis are often seen

The *Embryonal Carcinomas* present a less uniform picture than do the seminomas The undifferentiated type of embryonal carcinoma is composed of large cells with indistinct borders and amphophilic cytoplasm The nuclei are large round or reniform and contain coarse chromatin clumps and multiple nucleoli Differentiation in the embryonal carcinomas is characterized by structures that resemble primi

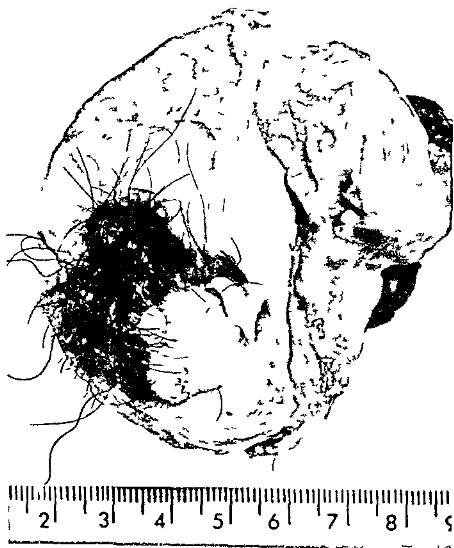


FIG 9 TERATOMA OF TESTIS Cystic spaces containing hair are reminiscent of dermoid cysts of the ovary but the tumors follow a malignant course (Courtesy of Dr T J Moran)

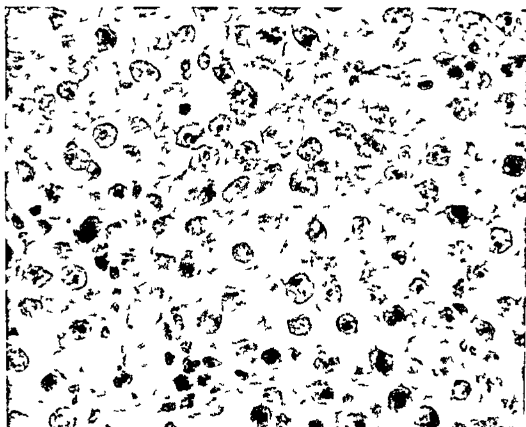


FIG 7 SEMINOMA OF TESTIS A higher magnification demonstrates distinct cell borders clear cytoplasm and distinct nucleoli × 435 (A F I P #100819) (Courtesy of Dr Frank J Dixon)

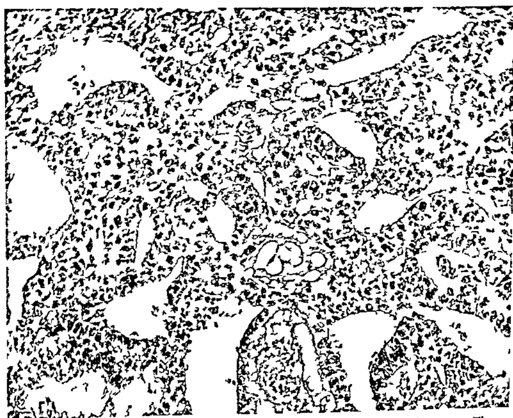


FIG 8 EMBRYONAL CARCINOMA OF TESTIS The cells have large nuclei with coarse chromatin The spaces formed are not true acini × 155 (A F I P #154376) (Courtesy of Dr Frank J Dixon)



FIG 11 CHORIOCARCINOMA OF TESTIS The large dark masses are syncytiotrophoblastic cells and they are intermingled with sheets or single cytotrophoblasts ×90 (A.F.I.P #93056) (Courtesy of Dr Frank J Dixon)

ferentiated and characteristic of certain tissues are common but organization of tissues to form organs such as bronchus intestine skin and bone can be seen (Figs 9 and 10) Maturity of tissues does not correlate with the clinical behavior of teratomas

Choriocarcinomas are composed of two types of cells the syncytial cells being applied to the surface of cytotrophoblastic masses to mimic placental tissue (Fig 11)

Combinations of the types described above create the confusion that exists concerning testicular neoplasms All of the combinations were divided into five groups on a histologic basis This also furnished a clinically useful grouping The teratoma in combination with choriocarcinoma or embryonal carcinoma makes these two less malignant but the seminoma the least malignant as a pure tumor when in combination does not alter the very malignant course of the others

I Seminoma pure
II Embryonal carcinoma pure or with seminoma
III Teratoma pure or with seminoma
IV Teratoma with either embryonal carcinoma or choriocarcinoma or both and with or without seminoma
V Choriocarcinoma pure or with either embryonal carcinoma or seminoma or both

Group I was most common (38%) Group IV was next (32%) Group II was third (20%) Group III was fourth (9%) and Group V was least frequent (1%)

Grossly the Group I tumors were the largest but they varied considerably in size The tumors replaced the testis and expanded the capsule without extending beyond it except in a small percentage of cases The tumors were firm bulged from the cut surface and were gray white with discernible foci of ischemic necrosis The Group II tumors were the smallest but the capsule was often invaded with recognizable extension to the epididymis or cord in one fourth of the group The cut surfaces were granular and gray white with extensive necrosis Group III tumors often replaced the testes but infrequently extended beyond the capsule Cystic spaces filled with fluid gave a honeycomb appearance to the cut surfaces (Fig 9) Cartilage and bone were often identified grossly Group IV tumors varied in size but tended to be large Cysts alternated with hemorrhagic necrosis on the cut surfaces Group V tumors were small and the testes were altered only slightly if at all in size and contour The sectioned tumors were hemorrhagic and necrotic except for a rim of preserved tissue

Autopsies on men who died of these tumors demonstrated in all cases a pattern of metastases to regional

tive epithelium (Fig 8) or mesoderm The structures formed by the primitive epithelium resemble acini and this has led to the mistaken diagnosis of adenocarcinoma Differentiation to form trophoblasts may also be seen in the embryonal carcinoma Extensive hemorrhagic necrosis is frequently seen in these tumors

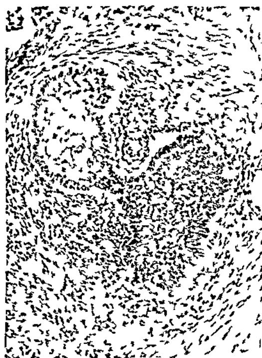
Teratomas contain derivatives of all three of the germ layers but their representatives are disorganized and may be difficult to recognize Cells that are dif



(a)



(b)



( )

Fig 10 Teratoma of testis Various types of tissue are found Respiratory epithelium with cartilage (*a*) bone (*b*) and a tooth bud (*c*) are represented × 200 (Courtesy of Dr Frank J Dixon)

Non-Germinal Tumors

The non germinal tumors comprise 3.5 per cent of the cases reported from the A F I P [9] Capsular fibromas and interstitial cell tumors numbering 14 and 12 respectively form the largest number There are 4 androblastomas and 2 adrenal cortical rest tumors Adenocarcinomas of the rete testis are rare Connective tissue tumors including sarcomas are infrequently reported as arising within the testis

Interstitial cell tumors vary from small to rather large tumors that compress the testis into a thin rim beneath the capsule They are rounded or oval and appear on section to be yellow or brown and homogeneous Microscopically the tumors are composed of polyhedral cells with pink granular cytoplasm (Fig 13) Granules of brown pigment are commonly found in the cytoplasm and crystalloids of Reinke are seen in one-fourth of the cases The nuclei are eccentrically situated in the cells and may be large and hyperchromatic The interstitial cells in the un involved testis are either normal or decreased in number or hyperplastic

Tumors of interstitial cells that occur before puberty may be recognized because of the resulting precocious sexual development After puberty an increase in androgens does not produce clinical signs and symptoms and the adult male presents with the complaint of a mass in the testis A review [67] of interstitial cell tumors cites 37 reported cases in adults and 9 cases in children Approximately 15 per cent of the patients had gynecomastia There was also loss of libido and testicular atrophy in these patients In 5 of the 37 adult patients the tumor was malignant None of the 12 cases in the A.F I P series was malignant.[70]

A frequent finding in children with the adrenogenital syndrome is rests of cells in the testis that resemble interstitial cells These cells are probably adrenal cortical cells Morphologically they cannot be specifically differentiated from interstitial cells but these rests do respond to cortisone as do adrenal cortical cells [110] The rests may be so extensive that they are grossly visible as brown tumors [5] the possibility must be considered that an unknown number of reported examples of interstitial cell tumor of the testis are actually adrenogenital syndrome with adrenal rests in the testis Hedinger [34] questions in a 37 year-old man with adrenogenital syndrome whether the testicular lesion is Leydig cells or adrenocortical tissue If the symptoms are not alleviated by removal of the tumor and if there are no crystalloids of Reinke in tumor cells there should be skepticism in accepting the lesion as a proven interstitial

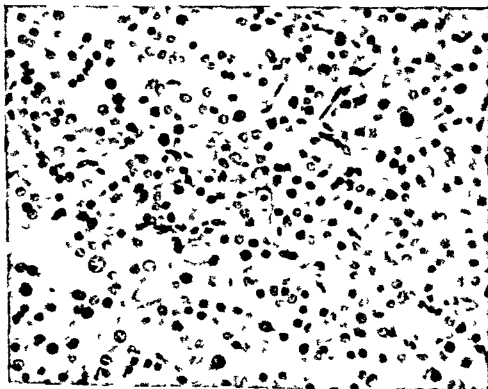


FIG 13 INTERSTITIAL CELL TUMOR OF TESTIS The tumor cells have pink granular cytoplasm The dark nuclei do not indicate a malignant course × 350 (A.F I P #90750) (Courtesy of Dr Frank J Dixon)



FIG 12 INTERSTITIAL CELL HYPERPLASIA This indicates a poorer prognosis in patients with germinal tumors of the testis × 125 (A F I P #106389) (Courtesy of Dr Frank J Dixon)

lymph nodes and distant spread to lungs liver brain bones and kidney Group I tumors had a five-year mortality of 10 5 per cent calculated from time of operation Group II tumors 64 5 per cent Group III tumors 29 2 per cent Group IV tumors 52 2 per cent and Group V tumors a 100 per cent five year mortality[21] The highest mortality with testicular tumors occurs in the first two years The A F I P series of cases was treated by surgery followed by radiation except for a small percentage of patients Retroperitoneal node dissection seemed beneficial to patients with tumors in Group II and Group III and radiation was especially beneficial to patients with Group I tumors

Certain clinical and pathologic findings can be related to prognosis of patients with germinal tumors of the testis If pain is a symptom the prognosis is worse if the tumor is beyond the capsule of the testis cure is less likely Vascular invasion by the tumor is a sign of poor prognosis If a lymphoid stroma is present in Group I tumors there is a better rate of cure than if the tumor lacks this Interstitial cell hyperplasia is associated with a poorer prognosis in all the groups (Fig 12) For example in Group I tumors a 32 per cent two year mortality was reported if interstitial cell hyperplasia was evident and the mortality in two years was only 5 per cent if this was absent

Gonadotropins of either the chorionic or pituitary type are found in the urine of many patients with testicular tumors Twombly[106] found chorionic gonadotropins in the urine of 35 of the 65 men studied and 18 of the men excreted pituitary gonadotropins The histology of the testicular tumors could not be predicted by these findings the tumors being of various types In the series reported by Dixon and Moore[1] only one-third to one half of the patients were studied for gonadotropin excretion and the type of gonadotropin was not ascertained Of the patients studied 13 per cent of Group I and approximately 25 per cent of Groups II III and IV excreted gonadotropins

The presence of urinary gonadotropin is a bad prognostic sign Detection of chorionic gonadotropin is especially ominous for it is thought to originate in the tumor and its persistence after treatment indicates the presence of tumor Why pituitary gonadotropin should be formed is problematical but it too is an unfavorable sign Simultaneous excretion of both types may occur[32] Estrogens have been excreted in excess by men with testicular tumors[106] and a low androgen excretion has been reported[32]

tumors They were yellow lobulated and demarcated from the adjacent testis The tubular type were tiny yellow round or oval nodules in testes that were often abnormal as a result of cryptorchidism or as a result of other tumors All of the androblastomas followed a benign course

It must be added that many of the lesions designated as tubular adenomas are not true neoplasms but are merely remnants of immature tubules [7] These are seen most frequently in cryptorchid testes and are one of the lesions that have mistakenly contributed to the stated incidence of tumors coincident with cryptorchidism

Of various miscellaneous tumors that have been described in the testis those designated as *granulosa cell tumors* are endocrinologically active One such was reported as estrogen producing by clinical and laboratory evaluation Another report of a granulosa cell tumor of the testis was in a 21 year old man who had many anomalies this patient also had gynecomastia [16]

While it is doubtful that *tumors of Sertoli cells* occur in man these tumors are common in male dogs Thirty three cases of this type of tumor were found in a series of 177 canine testicular tumors [86] Sertoli cell tumors may be recognized because of the hyperestrinism they produce The most obvious signs of this are sexual attractiveness to other male dogs hypertrophy of mammary papillae and focal hairlessness Sertoli cell tumors are of varying size tending to be small and are white or pale brownish yellow Microscopically the basic pattern is that of tubules divided by connective tissue but there is variation in the cells lining the tubules and variation in the amount of stroma Cancerous characteristics are recognized by the closely packed dark nuclei cellular pleomorphism frequent mitoses and extension of tubular cells into connective tissue Histogenetically the tumors appear to arise from tubules Spermatogonia may persist in tubules of the tumor Leydig cell rests are also occasionally found between tubules That Sertoli cell tumors produce estrogens seems established Clinical and pathologic change similar to those produced by estrogen are reversed by removal of the tumor and recurrence of the changes follows regrowth of tumor Estrogenic substances are recoverable from tumor tissue [49] and histochemical studies point to the tumor cells as the probable site of origin of the substances [86]

PROSTATE

Anomalies

Anomalies of the prostate are rare Absence of the gland is usually associated with major defects such as exstrophy of the bladder Hypoplasia of one side of the prostate occurs with other unilateral anomalies especially renal agenesis

Infections

Tuberculosis occurs in the prostate more commonly than elsewhere in the genital tract In autopsy studies 80 per cent to 90 per cent of tuberculous infections that involve the reproductive organs are in the prostate A series of autopsies on tuberculous patients revealed tuberculous prostatitis in 12 per cent [2] Tubercle bacilli may spread from the kidney to the prostate via the urinary tract but hematogenous dissemination from a pulmonary focus is the usual mechanism of spread The prostate thus receives the tubercle bacilli simultaneously with the liver spleen and meninges Grossly tiny foci or large caseous masses are seen in the prostate Microscopically caseous necrosis giant cells of the Langhans type epithelioid cells and lymphocytes comprise the classic tubercle Effective antibiotic therapy changes the picture to active healthy granulation tissue with little caseous necrosis [53]

Degenerative Disorders

It has been known for many years that development of the prostate gland is dependent upon the presence of the testes and that castration results in atrophy of the prostate The sensitivity of the prostate of immature laboratory animals to androgens is the basis for the bio assay of androgen containing drugs Androgen administration in infancy and childhood results in prostatic enlargement The presence in the prostate of the enzyme acid phosphatase is related to androgen stimulation Scant amounts of the enzyme are in the gland before puberty and large quantities post puberally It has been reported that the prostate of an infant with pseudoprecocious puberty due to adrenocortical hyperplasia contained acid phosphatase in amounts comparable to that seen in adults [3]

Hyperplasia of the prostate is commonly designated as benign hypertrophy a term both inaccurate and redundant A better term is hyperplasia which denotes an increase in number of cells and also by definition a benign condition Since nodules are formed nodular hyperplasia is an accurate and descriptive term Hyperplasia of the prostate occurs infrequently before the age of 50 years but thereafter its incidence rises with increasing age Three fourths of males over 80 have anatomical changes of hyperplasia though many have no symptoms at this age Clinical symptoms occur most frequently in men of 60 70 years of age The appearance of hyperplasia at about the age of 50 has led to speculation that decrease in androgen formation with a

cell tumor. There are a few cases reported in the literature that justify skepticism[67,101] if the criteria above are applied.

Tumors of interstitial cells are the only testicular neoplasm that can regularly be produced experimentally, but this is accomplished only in mice of certain strains.[8] Prolonged estrogen administration induces the tumors, and metastases occur from certain ones. The tumors produce androgens, as shown by the reversal of the effect on the prostate of the estrogens that were given to produce the neoplasm.[45] Histochemical studies[8] indicate this production of androgen. The absence of crystalloids of Reinke from interstitial cells of mice eliminates this criterion for identification of the tumors. Spontaneous interstitial cell tumors occur in dogs, the incidence in reports varying from 1 per cent to 80 per cent.[6] In one series of 88 Leydig cell tumors in dogs there was no evidence of endocrine activity by the tumor.[86] Horses also may develop interstitial cell tumors.

Androblastoma was the name applied by Teilum to a feminizing testicular tumor seen in a 53-year-old male.[104] The author considered the androblastoma as homologous to the arrhenoblastoma, which is typically a virilizing tumor arising in the ovary. Later[105] virilizing tumors of the ovary were included as androblastomas. Hormonal effect of this neoplasm depends upon which portion of the testicular blastema develops. If the Sertoli cell portion develops the tumor is estrogenic; if the Leydig cell portion develops it is an androgenic tumor. The endocrine effect of the tumor should correlate with the morphology, and in a small group of cases there is such a correlation. Both the feminizing tumor of Teilum's original report[104] and the androblastoma in the A.F.I.P. series[9] which was associated with gynecomastia had tubules in which Sertoli cells were seen. Also ovarian arrhenoblastomas of the tubular adenoma type may be manifested by symptoms suggestive of excessive estrogen formation. However, arrhenoblastomas are usually virilizing if they have prominent stroma, the stroma being tissue that is directed to Leydig cell differentiation.

Androblastomas in the A.F.I.P. series were classified as tubular, mixed, and diffuse stromal types. This may represent degrees of differentiation of the embryonic testis. The tubular type is the most differentiated, with tubules lined by small columnar cells. The diffuse stromal type is composed of spindle-shaped cells with scant cytoplasm and small dark nuclei. The mixed type is composed of combinations of the other two (Fig. 14). Grossly the mixed and diffuse types of androblastomas in the *A.F.I.P.* series were larger tumors on the average than the germinal

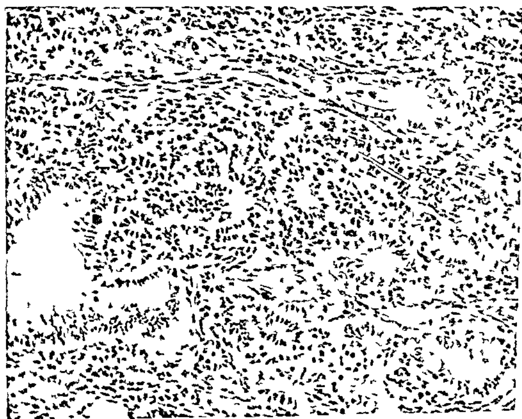


FIG. 14. ANDROBLASTOMA OF TESTIS, MIXED TYPE. The tubules are separated by a stroma of oval or spindle shaped cells. × 185. (A.F.I.P. #109373.) (Courtesy of Dr. Frank J. Dixon.)

do not Injection of androgens or estrogens has produced no definite beneficial effect Squamous metaplasia of ducts occurs as a result of estrogen administration

The first changes of nodular hyperplasia are seen in the stroma Both the connective tissue and smooth muscle increase in amount and later there is an accompanying growth of glandular tissue In an advanced stage the enlarged gland is rubbery and gray and there are grossly visible nodules bulging from the surface Within many nodules there are cystic spaces The middle and lateral lobes are the site of the nodule formation and the posterior lobe becomes a thin compressed rim of tissue Microscopically the typical nodule is composed of acini with separating stroma the peripheral acini are stretched into a crescent shape Fibromuscular stroma is of varying prominence Certain nodules are composed of smooth muscle with scant fibrous tissue and no acini These have been mistakenly called leiomyomas Lymphocytic infiltration is common in the enlarged gland and infarcts occur frequently In the infarcted areas squamous metaplasia in the ducts (Fig 16) simulates squamous cell carcinoma [75]

Calculi occur in the prostates of up to 20 per cent of older men The stones are typically small and multiple and vary in color from white or gray to brown Usually no clinical symptoms result from their presence and they are found incidentally at autopsy or surgery

Tumors

Adenocarcinoma is the only neoplasm that occurs with significant frequency in the prostate It is first seen after the age of 40 when androgens are diminishing and atrophy of epithelium begins The incidence of prostatic carcinoma increases steadily after this age with the peak incidence in the seventh decade Detailed studies of autopsy specimens of the prostate from elderly men have revealed carcinoma in as high as 48 per cent of the 80 89 age group and 80 per cent of the 90 99 group [44] These carcinomas had produced no symptoms The carcinoma arises in the posterior lobe where hyperplasia does not occur There is probably no relation between hyperplasia and carcinoma as study of early carcinomas indicates that the cancer originates from the atrophied epithelium [71]

Carcinoma of the prostate and nodular hyperplasia seem to have a similar endocrinological background [99] Histologic studies of the pituitary indicate increased activity of this organ in patients with carcinoma and in those with hyperplasia An excess



FIG 16 SQUAMOUS METAPLASIA IN PROSTATIC DUCT This occurs in areas of infarction ×240 (A F I P #218716) (Courtesy of Dr Frank J Dixon)

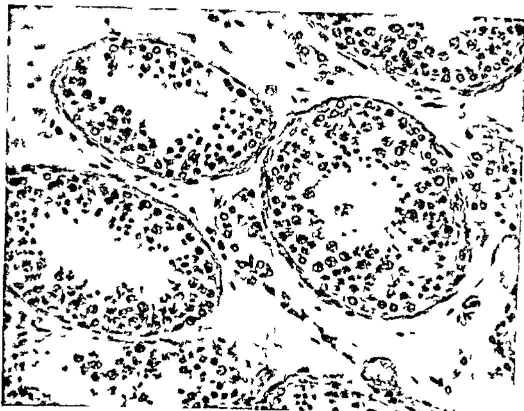


FIG 15 SPERMATOGENIC CELL HYPERTROPHY IN TESTIS OF PATIENT WITH CARCINOMA OF PROSTATE X 300 (Courtesy of Dr E R Fisher)

preponderance of estrogen is related etiologically to prostatic hyperplasia The idea of endocrine imbalance can be correlated with the occurrence of hyperplasia in the medial and lateral lobes only for these lobes are the parts of the prostate that have a counterpart in the female and are considered as ambisexual Jakobsen[50] found squamous metaplasia and epithelial hyperplasia in the ducts of prostates in which nodular hyperplasia was present The prostates of the control group where there was no nodular hyperplasia did not show the same changes in the ducts This was considered evidence of estrogen excess Sommers[95] described hypertrophy of spermatogenic cells (Fig 15) in 76 per cent of men with prostatic hyperplasia These testicular changes were cited as an indication of estrogen excess in patients with hyperplasia An increase in the pituitary gonadotropic basophils[99] was also discovered in these patients By analogy with the female the gonadotropic cells of the pituitary were thought to form an excess of follicle stimulating hormone However there is no clearly delineated evidence from studies of hormone excretion that sustain the inferences based on anatomic data

Animals do not develop the anatomical equivalent of hyperplasia with formation of nodules as it occurs in man and attempts to produce it by various hormone alterations have been unsuccessful Atrophy of the prostate and squamous metaplasia of ducts occur with estrogen administration

Isolated clinical observations relative to prostatic hyperplasia are interesting but furnish little insight into its etiology Eunuchs and castrates do not develop this condition Males with pituitary infantilism due to various pituitary lesions have atrophic prostates and these patients who have survived to over 40 years of age have no evidence of the nodules of hyperplasia Among Chinese men over 40 years of age only 6 per cent have nodular hyperplasia whereas 40 per cent of Caucasian men do The Buddhist law prohibits sexual intercourse after the age of 35 years but it is doubted that this can explain the infrequency of hyperplasia of the prostate in the Chinese for prostatic hyperplasia occurs among priests of the Catholic Church Negroes in this country develop prostatic hyperplasia with the same frequency although earlier than do whites

The effects of castration after prostatic hyperplasia has developed are not clear cut Those who advocated castration in the years prior to 1900 collected cases to prove its beneficial effect but comparison of results with untreated controls proves no significant clinical benefit from castration The effect on the microscopic picture is also indefinite Moore[73] indicates that perhaps a few patients respond to orchiectomy with atrophy of the prostate while others

interesting finding in a histochemical study is the decreased solubility of the ground substance especially of the basement membrane after estrogen treatment In the treated cancer of the prostate the basement membrane resists the spread of carcinoma[2]

Adrenalectomy for palliation of advanced prostatic cancer is effective in reducing the androgens to a lower point than does orchiectomy alone[3] The problem of caring for the adrenalectomized patient in part counterbalances the benefits of tumor regression Cortisone however has made possible the maintenance of the patient. Doses of cortisone up to 50 mg daily do not increase the androgen level[7] A description has been given of massive necrosis of the primary carcinoma of the prostate and its metastases after adrenalectomy[3]

SEMINAL VESICLES AND TUBULAR PASSAGES

Anomalies

Congenital abnormalities of the vas deferens and epididymis are frequent enough to be of practical significance to the physician who is concerned with fertility in the male Michelson[65] found 12 cases of anomalies in 900 subfertile men (1 3%) others have *found a similar or higher incidence Of 74 cases in* the literature with absent or aberrant vas 60 had defects of the epididymis Abnormalities were usually unilateral Defects of the testis were described in 58 and unilateral cryptorchidism was present in 30 Among Michelson s 12 cases three biopsied testes were normal though in the reviewed cases only 16 of 74 were reported as having bilateral normal testes Other series have recorded associated anomalies of kidney and seminal vesicles but not of the testes[5]

Infections

Infection in the seminal vesicles is usually gonococcal in origin and often accompanies a posterior urethritis and epididymitis Non specific infections may occur The sacculated body of the vesicles may be filled with a purulent exudate Retention of secretions may cause abscesses to form or may lead to a chronic condition Cysts may form in rare cases Tuberculosis may involve the seminal vesicles as a part of prostatic involvement

Infections in the ductus deferens especially those due to the gonococcus may spread and reach the epididymis and elicit a profuse neutrophilic reaction with edema and hyperemia. Chronic inflammation and scarring result in obstruction and azoospermia if the process is bilateral The epididymis may be palpable as thickened tubes in the scrotum Microscopically the obliterated duct may have no recognizable lumen being replaced by dense scar tissue

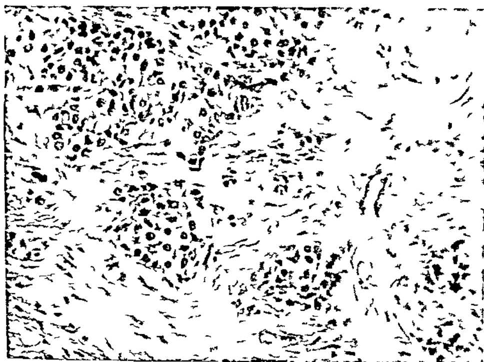


Fig 18 Adenocarcinoma of prostate after estrogen therapy Connective tissue is prominent and nuclei are small and dark. × 300 (Courtesy of Dr E R. Fisher)



Fig 17 Adenocarcinoma of prostate The tumor forms acini the nuclei are bland but stroma is extensively invaded × 185 (A F I P #218716) (Courtesy of Dr Frank J Dixon)

of estrogens is the hormonal aberration associated with both of these diseases It is difficult to reconcile this with the beneficial effects of estrogen therapy on carcinoma of the prostate

The carcinoma may be palpable as a firm nodule or the entire prostate gland may be hard Late in the course of the disease the gland is fixed in the pelvis to all adjacent structures When sectioned the tumor is seen to be gritty and hard and the cut surface is gray with interspersed pale yellow areas The histology of the tumors varies but acinar formation is generally evident very few of the tumors being completely undifferentiated The usual adenocarcinoma is composed of moderately uniform glands (Fig 17) and the cells appear disarmingly benign Invasion of perineural lymphatics can be found in nearly all prostatic carcinomas and the presence of invasion resolves any doubt as to malignancy Metastases from prostatic cancer involve regional lymph nodes vertebrae and bones of the pelvis Spread of tumor to the lungs and liver also occurs The perivertebral plexus of veins is an important route of dissemination

Carcinoma of the prostate forms large quantities of acid phosphatase the enzyme being demonstrable in tumor cells by appropriate histochemical technique Eighty per cent or more of patients with metastases have elevated serum acid phosphatase A level above 1 0 Bodansky unit is practically pathognomonic of carcinoma metastatic from the prostate A favorable response to treatment of the cancer is accompanied by a fall in serum acid phosphatase with recurrence of tumor the phosphatase is again increased The level of serum aldolase [5] may also be useful as an indicator of response to therapy

Experimental work with dogs has pointed to dependence of prostatic epithelium on androgens [17, 18] A result of these experiments was a trial administration of estrogen to men with prostatic carcinoma The results were impressive Orchiectomy has also been used As a palliative device both estrogens and castration have been effective Some patients with histologically proven carcinoma of the prostate who have been treated in either of these two ways have later died from other causes in several of these cases at autopsy no cancer was found Such cases are rare but they suggest that cure resulted from altering the hormonal environment of the tumor

The histologic changes in prostatic carcinomas after response to orchiectomy or administration of estrogens include pyknosis of nuclei and vacuolization of cytoplasm Mitoses are greatly reduced in number Later only a few scattered strands of tumor cells may be seen in fibrous tissue (Fig 18) An

is round with fine chromatin and may be displaced eccentrically by vacuoles This tumor is benign

Lipomas are the most common of the benign connective tissue tumors They are yellow vary greatly in size and are composed of mature fat cells *Fibromas neurilemomas* and *leiomyomas* occur as firm gray nodules differentiated by their microscopic appearance

Sarcomas occur rarely but are seen more often in children and young adults They may be of various histologic types Rhabdomyosarcomas leiomyosarcomas and fibrosarcomas have been described

Adrenal cortical rests occur frequently along the cord The incidence varies with the series but is as high as 76 per cent [10] These rests have an organoid structure with zones resembling the normal adrenal The rests of Leydig cells sometimes seen about the testis do not have an organoid structure They are often around nerve fibers and occasionally contain crystalloids of Reinke

PENIS SCROTUM AND URETHRA

Anomalies

While anomalies of the penis and scrotum are not common when they do occur multiple defects in the same individual are seen more often than a single defect Some of the rare anomalies of the penis are absence of the organ abnormal location double penis and double glans Phimosis occasionally appears as a congenital malformation Epispadias a dorsal defect in the penis with the urethral opening on the upper surface is rare Hypospadias is much more common In this condition the degree of maldevelopment varies the defect consisting merely of an opening at the frenum the most usual occurrence or an opening elsewhere along the urethra on the under surface of the penis When perineal hypospadias is present other defects including abnormalities of the utricle are commonly found The urethra may be atretic or double or more important may be the site of valves which obstruct the flow of urine These valves may go unrecognized as they permit retrograde passage of catheter or sound The scrotum may be completely bifid resembling the labia of the female or the division may be less marked One or both sides may be rudimentary

Traumatic Injuries

Severe traumatic damage to the penis and scrotum occur infrequently Serious injuries do occur however with amputation of penis and avulsion of the scrotum Following the classical straddle injuries with damage to the urethra infection often due to extravasation of urine can be a complication Gangrene infection and severely destructive sequelae may follow trauma

Infections

The venereal diseases except gonorrhea typically have a recognizable lesion of the penis The primary lesion of *syphilis* the chancre is a superficial ulcer overlying a larger indurated button like area The ulcer has a sharp margin and a red smooth base Histologically the infiltrate of inflammatory cells consists of lymphocytes plasma cells and macrophages with neutrophils superficially at the base of the ulcer Capillary and fibroblastic proliferation are present and peripherally the vessels are accentuated by the perivascular pattern of the infiltrate Spirochetes are demonstrable by various techniques Secondary and tertiary syphilis ordinarily do not involve the penis and scrotum except for the widespread rash in the secondary stage

Chancroid is an acute infectious disease usually spread by sexual contact and caused by a short gram negative bacillus *Hemophilus ducreyi* There may be several ulcerations The typical ulcer has a ragged soft border and has a dirty looking gray exudate on its base The ulcers may become large and deep Microscopically three indefinite zones can be recognized in the wall of an ulcer Degenerating neutrophils fibrin red blood cells and necrotic debris form the first layer In the second layer the granulation tissue is edematous and neutrophils are conspicuous in and about the capillaries Swollen proliferating endothelial cells are present and thrombi may be seen in the capillaries The third zone is composed of densely packed lymphocytes and plasma cells The characteristic histologic feature of this disease is the vasculitis of the second zone Within several weeks enlarged inguinal lymph nodes develop in almost half the cases A vasculitis similar to that seen in the ulcer is also seen in the abscessed lymph nodes The widespread change in the nodes results in capsular involvement and interadherence of nodes The histology of the ulcer is sufficiently characteristic so that biopsy is recommended as a diagnostic tool [89] Identification of the organism in smears however gives the positive diagnosis

Granuloma inguinale tends to remain localized in the skin and subcutaneous tissues It usually does not spread to lymph nodes though it persists as a chronic disease Infrequently it manifests itself as a systemic infection with widespread foci It is caused by an organism called the Donovan body The ulcer seen on the penis or inguinal area may be quite large with a red granular floor The edges are rolled and overhanging With chronicity growth of granulation tissue may give a nodular appearance to the floor of the ulcer Microscopically the disease

The lower portion of the epididymis is most frequently involved and the adjacent ductus deferens is also often affected

The importance of infection as a cause of sterility is emphasized by the figures given by surgeons who attempt to correct the obstruction There must be innumerable other instances in which no remedy is sought O Conor[81] attributed 61 of 123 cases of azoospermia to bilateral epididymitis Oligospermia may also be due to partial obstruction of the duct and instances have been cited where a low sperm count reached normal levels after reparative procedures

Tuberculosis of the epididymis is often a part of genito-urinary infection The thickened masses of tubes are filled with cheesy material and multiple sinuses may result The histologic picture is the same as that of tuberculosis elsewhere with identification of the acid fast organism the essential diagnostic point Involvement of the testicle may occur secondarily but it is often completely spared

Degenerative Disorders

The seminal vesicles like the prostate are dependent upon stimulation by the testis for growth and maintenance of size and functions They atrophy after castration and regrowth to normal size follows substitution therapy

Tumors

Tumors involving the seminal vesicles are rare unless one considers secondary metastases from carcinoma of the prostate In a recent review[20] only 26 cases of primary carcinoma two sarcomas and three myomas are cited The carcinomas are usually adenocarcinomas and metastases occur frequently

The epididymis and spermatic cord structures rarely give rise to tumors except those of connective tissue origin This is true if the most common neoplasm in this area the *adenomatoid tumor* is considered to be of connective tissue origin This supposition however may not be correct since origin of the adenomatoid tumor is not known Remnants of Wolffian or Mullerian ducts have been indicted and blood vessel and mesothelial cells have been considered as possible sources Dixon and Moore[20] cite evidence against acceptance of any of these and favor the noncommittal term adenomatoid tumor

The adenomatoid tumor is small 1 0 or 2 0 cm in diameter and usually attached to the lower end of the testis or epididymis It is rounded encapsulated firm and gray white The microscopic appearance varies and the stroma may be so abundant that the nature of the tumor is not recognized Within the stroma are cords or spaces the cells that form them have pink cytoplasm with vacuoles (Fig 19) The vacuoles are neither fat nor mucus The nucleus

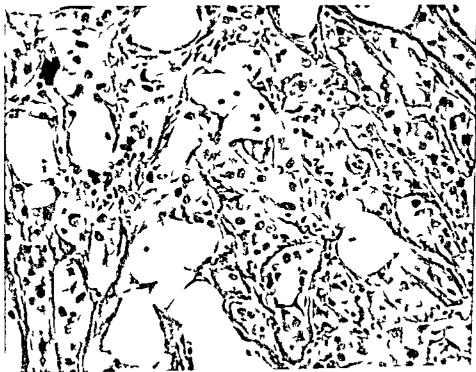


FIG 19 ADENOMATOID TUMOR OF SPERMATIC CORD The large cells have pink cytoplasm The vacuoles contain neither mucus nor fat × 260 (A F I P #114735) (Courtesy of Dr Frank J Dixon)

hemorrhagic or purulent It has a high specific gravity usually over 1 020 classifying it as an exudate The sac lining may be smooth gray and glistening resembling the normal tunica it may be loculated or thickened with gray and yellow plaques The pressure of the fluid on the testis may cause severe atrophy

Varicocele is a dilatation of veins of the pampini form plexus It occurs in varying degrees of severity and with perhaps a liberal interpretation of the definition the incidence has been placed as high as 10 per cent Varicocele is more frequently found on the left side and is seen in young men It has been described on physical examination as resembling a 'bag of worms The deleterious effect unless the varicocele is quite large is probably mainly psychological Resection of the mass of dilated veins has been beneficial in some cases of sterility [8]

Tumors

Condyloma accuminatum is a tumor like prolifera tion probably of viral etiology that results in papillomatous growths about the glans and in the sulcus A single mass or multiple extensive masses are encountered Histologically great thickening of the prickle cell layer is evident with formation or branching frond like growths The dermis contains a chronic inflammatory infiltrate

Papillomas composed of squamous cells and dif ferent in origin from the condyloma accuminatum in that they are true tumors are occasionally en countered They are innocuous and benign

The malignant counterpart of the papilloma is the *squamous cell carcinoma* It is seen usually in the older age group but is actually infrequent in this country where circumcision is commonly practiced One of the most interesting aspects of carcinoma of the penis is that it can be prevented by early cir cumcision Among Jewish males who are circumcised by ritual eight days after birth only one authentic case of carcinoma of the penis has been found There is evidence that circumcision at puberty is much less effective in cancer prophylaxis Phimosis and infec tions of the penis have been found so often with cancer of the penis that they are considered as etiologically related Smegma produces cancer when applied experimentally to mice [8]

Carcinoma is most frequently seen about the glans or in the sulcus as elevated indurated nodules that later ulcerate or as extensive papillary growths that also eventually ulcerate Microscopically the tumor is rather well differentiated the cells having inter cellular bridges and forming keratin masses Metas tasis occurs to regional lymph nodes in approximately one third of the patients Those tumors however that have primarily a papillary form rarely metas tasize The percentage of cures of patients with metastases is quite low

Squamous cell carcinoma of the scrotum was the first cancer to be recognized as an occupational dis ease It occurred in chimney sweeps following chronic exposure to soot containing carcinogenic hydrocar bons Many patients with carcinoma of the scrotum have a history of chronic exposure to oils paraffin creosote or tar [1] Leukoplakia often precedes devel opment of the carcinoma The cancer resembles the squamous cell carcinomas of the penis Metastases are to inguinal lymph nodes and are rarely to distant organs

Bowen s disease has been considered as synony mous with carcinoma *in situ* It may occur anywhere on the body as well as on the penis and appears as a red scaly patch that may persist for many years It consists microscopically of thickened epithelium with a thin superficial layer of parakeratotic cells Beneath this layer the cells have large nuclei Mitoses are numerous and multinucleated cells are present Focal keratinization occurs This lesion may after many years become invasive

Erythroplasia of Queyrat is seen on the glans penis as reddish raised plaques with smooth surfaces and rolled margins Microscopically long rete pegs are present Between the pegs the stratum corneum and stratum granulosum are thin Mitoses are frequent but the cells are uniform in size and shape Pro-gression to invasive cancer has been described as occurring over a period of several years This has been considered by some as evidence that Erythro-plasia of Queyrat is carcinoma *in situ* Histologically however it does not have the nuclear changes and disarray of cells that are seen in carcinoma *in situ*

Adenocarcinomas of Cowper s gland and glands of Littre are quite rare Carcinomas of apocrine glands may extend to surface epithelium and appear as extra mammary Paget s disease *Melanomas* of the penis have been described and 11 are cited in a recent review A nevus on the scrotum must al ways be viewed with alarm Nearly all will show areas of junctional activity with pigmented cells in the epidermis A transition to a malignant melanoma may be seen In the latter there are sheets of cells with large nuclei containing coarse chromatin and typically the cytoplasm contains melanin pigment The tumors are highly malignant and metastasize widely *Sarcomas* arising in the penis and scrotum do occur though rarely

Within the urethra only two types of growth are of importance papillomas of transitional epithelium similar to those seen so commonly in the bladder and carcinoma most frequently of the squamous cell type The papillomas are soft fronds covered with transitional epithelium and may be extensive enough to cause obstruction The carcinomas are most often

can be recognized with specificity The pathognomonic feature is the scattered large mononuclear cells that contain the organisms The nucleus of the monocyte is often eccentric and clusters of the round or rod like bodies are found in cystic spaces in its cytoplasm The closed safety pin appearance of the organism is seen best with silver stains Other microscopic features are the minute abscesses formed by clusters of neutrophils many plasma cells with few lymphocytes and epithelial proliferation at the margin of the ulcer

Lymphopathia venerum (lymphogranuloma venereum) is caused by a filterable virus of the psittacosis group The lesion consists of a superficial ulcer with *regional lymphadenopathy The latter may be the* most conspicuous manifestation of the disease The skin lesion is usually on the penis it is not tender and is sharply outlined Histologically the floor of the ulcer consists of granulation tissue infiltrated with *plasma cells and* lymphocytes and a superficial layer of neutrophils Epithelial hyperplasia is seen at the margin The involved lymph nodes are seen several weeks later and become matted with eventual break down and sinus formation in many of them Microscopically *the centers of the abscesses are necrotic* with disintegrating neutrophils while macrophages palisade at the periphery Confluence of necrotic foci creates the typical stellate pattern Even with healing the pattern tends to persist Chronicity with secondary infection may lead to *scarring of the penis* The resulting lymphatic obstruction may lead to elephantiasis

There are other infectious processes that involve the penis *Herpes progenitalis* is seen as clusters of *tiny vesicles that rupture to form small ulcers* *Fusospirochetosis* of the penis is often secondary to previous infections and causes superficial ulcers that have a grayish coating and a fetid odor

Gonorrhea is caused by *Neisseria gonorrhoeae* and in the male *involves primarily* the anterior urethra The meatus is pouting and red with congestion of the mucosa *Ulcerations of the mucosa* occur and involvement of the periurethral gland produces a copious discharge The discharge contains many neutrophils in which can be found the gram negative diplococci The microscopic changes in the tissues are those of acute *infection with vascular dilatation* edema and neutrophilic exudation The formation of scar tissue causes strictures but these occur less frequently with current treatment Surgery and trauma now more commonly cause strictures than *does gonorrhea* Extension of the infection into the prostate seminal vesicles and epididymides is a damaging sequela

Various other conditions non venereal in origin may affect the penis and scrotum *Pemphigus varicella* and *psoriasis* are diseases of widely different nature that may incidentally involve the external genitalia

Tinea cruris frequently infects the *scrotum and* is caused by fungi either *epidermophyton trichophyton* or *microsporum* Itching reddish brown rings or patches with elevated borders are seen The organism can be recognized in *scrapings soaked in* sodium hydroxide Scales or vesicles are seen when the lesion is biopsied Rarely can one see the fungi in sections stained with hematoxylin and eosin though they may be seen with the periodic acid Schiff stain

Degenerative Disorders

Balanitis xerotica obliterans is a chronic progressive process that appears as firm whitish patches on the glans and prepuce It may follow circumcision and may eventually stenose the urethral meatus The *etiology is unknown It has been likened* however to a more widespread process *lichen sclerosus et atrophicus* Microscopically the epidermis is thin with flattened rete pegs and the dermis is homogeneous and dense in its superficial part Deeper in the dermis *there are* lymphocytes and histiocytes and *the intima* of the vessels is thickened

Peyronie s disease occurs in older men with the peak incidence in the early fifties Gray fibrous nodules occur in the sheaths of the corpora cavernosa *frequently in the septum on the dorsal surface* of the penis and near the glans Microscopically dense fibrous tissue is seen that resembles keloid Stout [103] considers Peyronie s disease a fibromatosis comparable to desmoid tumor or Dupuytren s contracture The nodules do not become malignant

Disturbance of Flow

Hydrocele is an accumulation of fluid in the tunica vaginalis the portion of peritoneum that is carried into the scrotum by the testis Hydrocele may be transient as with *gonococcal epididymitis* mumps orchitis or most commonly with trauma These cases generally resolve spontaneously *Persistent* hydrocele is usually of unknown etiology but is related to stasis The occasional hydrocele that follows *herniorrhaphy* is an example of this influence Estimates place the incidence of hydrocele as high as 1 per cent of adult *males Hydrocele presents as* an ovoid mass from which the testis is separable and which displaces the testis downward and posteriorly Congenital pinching off of segments of the processus vaginalis can result in fluid accumulations *located higher* in the cord and apart from the scrotum The quantity of fluid may vary from a few cubic centimeters *to several hundred* cubic centimeters The fluid may be clear and amber or dark

38 Heller C G and Nelson W O 1948 Classification of male hypogonadism and a discussion of the pathologic physiology diagnosis and treatment J Clin Endocrinol 8 345 66
39 Heller C G Nelson W O Hill I B Henderson E Maddock W O Jungck E C Paulson C A and Mortimore G E 1950 Improvement in spermatogenesis following depression of the human testis with testosterone Fert & Steril 1 415-22
40 Heller C G Paulson C A Mortimore G E Jungck E C and Nelson W O 1952 Urinary gonadotrophins spermatogenic activity and classification of testicular morphology—their bearing on the utilization hypothesis Ann N Y Acad Sci 55 685 702
41 Hempelmann L H Lisco H and Hoffman J G 1952 The acute radiation syndrome a study of nine cases and a review of the problem Ann Int Med 36 281 510
42 *Hinman F Jr* 1955 *Optimum time for orchiopexy* in cryptorchidism Fert & Steril 6 206 14
43 —— 1955 Spermatogenesis following experimental testicular ischemia Fert & Steril 6 443 9
44 Hirst A E and Bergman R T 1954 Carcinoma of the prostate in men 80 or more years old Cancer 7 136-41
45 Hooker C W and Pfeiffer C A 1942 The morphology and development of testicular tumors in mice of the A strain receiving estrogens Cancer Research 2 759 60
46 Howard R P Sniffen R C Simmons F A and Albright F 1950 Testicular deficiency a clinical and pathologic study J Clin Endocrinol 10 121 86
47 Huggins C and Clark P J 1940 Quantitative studies of prostatic secretion II The effect of castration and of estrogen injection on the normal and on the hyperplastic prostate gland of dogs J Exp Med 72 747 62
48 Huggins C Masina M H Eichelberger L and Wharton J D 1939 Quantitative studies of prostatic secretion I Characteristics of the normal secretion the influence of thyroid suprarenal and testes extirpation and androgen substitution on the prostatic output J Exp Med 70 543 56
49 Huggins C and Moulder P V 1945 Estrogen production by Sertoli cell tumors of the testis Cancer Research 5 510 14
50 Jakobsen A H I 1951 The influence of oestrogenic hormones on the prostate Acta Path et Microbiol Scand 29 419 25
51 Kennaway E L and Kennaway N M 1946 Social distribution of cancer of the scrotum and cancer of the penis Cancer Research 6 49 53
52 Keys A et al 1950 The Biology of Human Starvation The University of Minnesota Press Minneapol
53 Klatskin G Salter W T and Humm F D 1947 Gynecomastia due to malnutrition I Clinical studies Am J Med Sci 213 19 30
54 Klinefelter H F Reifenstein E C and Albright F 1942 Syndrome characterized by gynecomastia aspermatogenesis without a Leydigism and increased excretion of follicle stimulating hormone J Clin Endocrinol 2 615-27
55 Leuchtenberger C Leuchtenberger R Schrader F and Weir D R 1956 Reduced amounts of desoxyribose nucleic acid in testicular germ cells of infertile men with active spermatogenesis Laboratory Investigation 5 422-40
56 Liebow A A Warren S and DeCoursey E 1949 Pathology of atomic bomb casualties Am J Path 25 853 1027
57 *Lloyd C W and Williams R H* 1948 Endocrine changes associated with Laennec s cirrhosis of the liver Am J Med 4 315-27
58 Long M E and Engle E T 1952 Cytochemistry of the human testis Ann N Y Acad Sci 55 619 28
59 Loring W E and Vandiviere H M 1956 The treated pulmonary lesion and its tubercle bacillus I Pathology and pathogenesis Am J Med Sci 232 20 29
60 Lynch K M 1952 Recovery of the rat testis following estrogen therapy Ann N Y Acad Sci 55 734-41
61 MacLeod J 1952 Certain concepts in human male fertility J Urol 67 19 26
62 MacLeod J and Hotchkiss R S 1941 Effect of *hyperpyrexia upon spermatozoa counts in men* Endocrinology 28 780 84
63 Maddock W O Epstein M and Nelson W O 1952 The assay of urinary estrogens as a test of human Leydig cell function Ann N Y Acad Sci 55 657 73
64 Maddock W O and Nelson W O 1952 The effects of chorionic gonadotropin in adult men increased estrogen and 17 ketosteroid excretion gynecomastia Leydig cell stimulation and seminiferous tubule damage J Clin Endocrinol & Metab 12 985-1014
65 Mason K E 1933 Differences in testis injury and repair after vitamin A deficiency vitamin E deficiency and inanition Am J Anat 52 153 239
66 McCullagh E P and Schaffenburg C A 1952 The role of the seminiferous tubules in the production of hormones Ann N Y Acad Sci 55 674 84
67 Melicow M M Robinson J N Ivers W and Rainsford L K 1949 Interstitial cell tumors of the testes J Urol 62 672 93
68 Michelson L 1949 Congenital anomalies of the ductus deferens and epididymis J Urol 61 384 90
69 Mills R G 1919 The pathological changes in the testes in epidemic pneumonia J Exper Med 30 505 29
70 Moore C R and Oslund R 1924 Behavior of *testes in transplantation experimental cryptorchidism* vasectomy scrotal insulation and heat application Endocrinol 8 493 508
71 Moore R A 1935 The morphology of small prostatic carcinoma J Urol 33 224 34
72 —— 1937 Tuberculosis of the prostate gland J Urol 37 373 83
73 —— 1947 Endocrinology of Neoplastic diseases Oxford University Press New York pp 194 212
74 Morrione T G 1944 Effect of estrogens on the testis in hepatic insufficiency Arch Path 37 39 48
75 Mostofi F K and Morse W H 1951 Epithelial metaplasia in prostatic infarction Arch Path 37 39 48
76 Mulinos M G and Pomerantz L 1941 The re*productive organs in malnutrition Effect* of chorionic gonadotropin upon atrophic genitalia of underfed male rats Endocrinol 29 267 75
77 Munson P L Goetz F C Laidlaw J C Harrison J H and Thorn G W 1954 Effect of adrenocortical steroids on androgen excretion by adrenalectomized orchiectomized men J Clin Endocrinol & Metab 14 495-508

in the posterior urethra and frequently are in patients who give a history of urethral stricture Eighty eight per cent of the tumors in the urethra are squamous cell type and 12 per cent are glandular or transitional [6] The tumors are usually poorly differentiated and infiltrate widely Metastases occur early

BIBLIOGRAPHY

1 Albert A Underdahl L O Greene L F and Lorenz Nona 1953 Male hypogonadism II Classification Proc Staff Meetings Mayo Clinic 28 557 67

2 Arendt J A 1954 Role of the ground substance in atrophy of normal and malignant prostatic tissue following estrogen administration and orchiectomy J Clin Endocrinol & Metab 14 1113 25

3 Ashbel R Cohen R B Seligman A M 1951 *Histochemical demonstration of ketosteroids in normal and neoplastic testes* Endocrinology 49 265 81

4 Baker J W and Evoy M M 1942 Insult to the testicle in herniorrhaphy Surg Gynec & Obst 75 285-8

5 Baker R Gowan D Huffer J and Cason J 1953 Biologic titration of diethylstilbestrol against activity of prostatic cancer serum aldolase J Clin Endocrinol 13 383 91

6 Ballew J W and Masters W H 1954 Mumps a cause of infertility I Present considerations Fert & Steril 5 536-43

7 Baumrucker G O 1946 Incidence of testicular pathology Bull U S Army Med Dept 5 312 14

8 Bonser G M 1944 Mammary and testicular tumours in male mice of various strains following oestrogen treatment J Path & Bact 56 15 26

9 *Bunge R G and Bradbury J T 1956* Genetic sex chromatin test versus gonadal histology J Clin Endocrinol & Metab 16 1117 19

10 Burt A S Reiner L Cohen R B and Sniffen R C 1954 Klinefelter's syndrome report of an autopsy with particular reference to the histology and histochemistry of the endocrine glands J Clin Endocrinol & Metab 14 719 28

11 Cabot Case of the Massachusetts General Hospital 1955 New Eng J Med 253 926-31

12 Cattell R B and Anderson C 1931 End results in the operative treatment of inguinal hernia New Eng J Med 205 430 32

13 Changus G W Williams W L and Fennell R H 1950 51 Prostatic phosphatase in an infant with adrenogenital syndrome J Nat Cancer Inst 12 255

14 Charny C W 1952 Discussion of Lynch K M and Scott W W The Sertoli cells as related to age of man and experimental alteration of the pituitary gonad axis in the animal Fert & Steril 3 35 48

15 Charny C W Conston A S and Meranze D R 1952 Development of the testis a histologic study *from birth to maturity with some notes on abnormal* variations Fert & Steril 3 461 79

16 Cohen J and Diamond I 1953 Leontiasis ossea slipped epiphyses and granulosa cell tumor of testis with renal disease Arch Path 56 488 500

17 de la Balze F A Arrillage F C Irazu J and Mancini R E *1952* Klinefelter's *syndrome a study* of five cases Histophysiologic basis for a pathogenic interpretation J Clin Endocrinol & Metab 12 1426-44

18 de la Balze F A Mancini R E Bur G E and Irazu J 1954 Morphologic and histochemical changes produced by estrogens on adult human testes Fert & Steril 5 421 36

19 Deming C L and Clarke B G 1953 Torsion of the spermatic cord J A M A 152 521 2

20 Dixon F J and Moore R A 1952 Tumors of the Male Sex Organs Armed Forces Institute of Pathology Washington D C

21 ——— 1953 Testicular tumors A clinicopathologic study Cancer 6 427 54

22 Engberg H 1949 Investigations on the endocrine function of the testicle in cryptorchidism Proc Roy Soc Med 42 645-51

23 *Franks L M 1953 Structural changes in prostatic* cancer after bilateral adrenalectomy Brit Med J 2 359-60

24 Gall E A 1947 The histopathology of acute mumps orchitis Am J Path 23 637 51

25 Gardner L I Sniffen R C Zygmuntowicz A S and *Talbot N B 1950* Follow up studies in a boy with mixed adrenal cortical disease Pediatrics 5 808 23

26 Gardner W U et al 1953 Hormonal Factors in Experimental Carcinogenesis in the Physiopathology of Cancer Paul B Hoeber Inc New York p 250

27 Gassner F X and Hill H J 1955 Testicular biopsy in the bull Effect on morphology of the testis Fert & Steril 6 290 301

28 Glass S J Edmonson H A and Soll S N 1940 Sex hormone changes associated with liver disease Endocrinology 27 749 52

29 Gross R E and Jewett T C Jr 1956 Surgical experiences from 1222 operations for undescended testes J A M A 160 634-41

30 Grumbach M M Van Wyk J J and Wilkins L 1955 Chromosomal sex in gonadal dysgenesis (ovarian agenesis) *relationship to male pseudohermaphroditism* and theories of human sex differentiation J Clin Endocrinol & Metab 15 1161 93

31 Hall H C 1912 Über Hodenatrophie nach parotitis epidemica Virchows Arch f path Anat 207 188 94

32 Hamburger C 1941 On the nature of gonadotrophin in cases of malignant tumors of the testis Acta Path et Microbiol Scandinav 18 457 84

33 Hamburger C and Godtfredsen E 1941 Studies on the excretion of androgenic substances and gon*adotrophin in cases of malignant tumors* of the testis especially seminoma Acta Path et Microbiol Scandinav 18 485 502

34 Harrison J H Thorn G W and Jenkins D 1953 Total adrenalectomy for reactivated carcinoma of the prostate New Eng J Med 248 86 92

35 Heckel N J and McDonald J H 1952 The effects of testosterone propionate upon the spermatogenic function of the human testis Ann N Y Acad Sci 55 725 33

36 Hedinger C 1954 Bilateral testicular tumors and congenital adrenogenital syndrome Leydig cell or adrenocortical tissue? Schweiz Ztschr Allg Path 17 743 50

37 Heller C G and Nelson W O 1945 Hyalinization of the seminiferous tubules associated with normal or failing Leydig cell function Discussion of relationship to eunuchoidism gynecomastia elevated gonadotrophins depressed 17 ketosteroids and estrogens J Clin Endocrinol 5 1 12

INDEX

Page numbers in italics indicate text figures and tables

78 Nelson R E 1950 Congenital absence of the vas deferens J Urol 63 176 82
79 Nelson W O 1948 The physiological basis of hypogonadism in the male M Clin North America 32 97 110
80 —— 1953 Some problems of testicular function J Urol 69 325 38
81 O Conor V J 1953 Mechanical aspects and surgical management of sterility in men J A M A 153 532-4
82 Pace J M and Cabot H 1936 Histologic study of twenty four cases of retained testes in the adult Surg Gynec & Obstet 63 16 22
83 Plunkett E R and Barr M L 1956 Cytologic tests of sex in congenital testicular hypoplasia J *Clin Endocrinol & Metab 16 829 30*
84 Pratt Thomas H R In discussion of W L Williams and C C Erickson 1956 A review of experimental studies upon the etiology of carcinoma of the cervix in humans and animals South Med Journal 49 101 9
85 Robinson J N and Engle E T 1949 Effect of neutron radiation on the human testis a case report J Urol 61 781 4
86 Scully R E and Coffin D L 1952 Canine testicular tumors with special reference to their histogenesis comparative morphology and endocrinology Cancer 5 592 605
87 Scully R E and Parham A R 1948 Testicular tumors II Interstitial cell and miscellaneous neoplasms Arch Path 46 229 42
88 Seckel H P G 1946 Precocious sexual development in children Med Clin North America 30 183 209
89 Sheldon W H and Heyman A 1946 Studies on chancroid I Observations on the histology with an evaluation of biopsy as a diagnostic procedure Am J Path 22 415 22
90 Simmons F A 1950 The treatment of male infertility Fert & Steril 1 193 8
91 —— 1952 Correlation of testicular biopsy material with semen analysis in male infertility Ann N Y Acad Sci 55 643 56
92 —— 1956 Human infertility New Eng J Med 255 1140 46 1186 92
93 Sniffen R C 1952 Histology of the normal and abnormal testis at puberty Ann N Y Acad Sci 55 609 18
94 Sniffen R C Howard R P and Simmons F A 1950 The testis II Abnormalities of spermatogenesis atresia of the excretory ducts Arch Path 50 285 95
95 —— 1951 The testis III Absence of germ cells sclerosing tubular degeneration male climacteric Arch Path 51 293 311
96 Sniffen R C Howard R P and Simmons F A 1954 The testis IV Idiopathic eunuchoidism with low FSH testicular changes secondary to lesions in or near the pituitary and secondary to estrogen therapy Arch Path 57 464 80
97 Sobel E H Sniffen R C and Talbot N B 1951 V Use of testicular biopsies in the differential diagnosis of precocious puberty *Pediatrics 8* 701 16
98 Sommers S C 1956 Testicular spermatogenic cell hypertrophy accompanying prostatic hypertrophy and cancer Am J Path 32 185 99
99 —— Endocrine changes with prostatic cancer *Cancer In press*
100 Staubitz W J Lent M H and Oberkircher O J 1955 Carcinoma of the penis Cancer 8 371 8
101 Staubitz W J Oberkircher O J and Blick M S 1953 Precocious puberty in a case of bilateral interstitial cell tumor of the testis J Urol 69 562 70
102 Stemmerman G N Weiss L Auerbach O and Friedman M 1950 A study of the germinal epithelium in male paraplegics Am J Clin Path 20 24 34
103 Stout A P 1953 Tumors of the Soft Tissues Armed Forces Institute of Pathology Washington D C
104 Teilum G 1946 Arrhenoblastoma—androblastoma Homologous ovarian and testicular tumors Acta Path et Microbiol Scand 23 252 64
105 —— 1949 Estrogen producing Sertoli cell tumors (androblastoma tubulare lipoides) of the human testis and ovary Homologous ovarian and testicular tumors III J Clin Endoc 9 301 18
106 Twombly G H 1947 The relationship of hormones to testicular tumors in Endocrinology of Neoplastic *Diseases Oxford University Press New York pp* 228 44
107 Weisel J 1899 quoted by Nelson A A in 1939 Accessory adrenal cortical tissue Arch Path 27 955 65
108 Werner C A 1950 Mumps orchitis and testicular atrophy I Occurrence *Ann Int Med 32 1066 74*
109 —— 1950 Mumps orchitis and testicular atrophy II A factor in male sterility Ann Int Med 32 1075 86
110 Wilkins L and Cara J 1954 Further studies on the treatment of congenital adrenal hyperplasia with cortisone V Effects of cortisone therapy on testicular development J Clin Endoc & Metab 14 287 96
111 Williams R H 1955 Textbook of Endocrinology W B Saunders Co Philadelphia pp 316 49